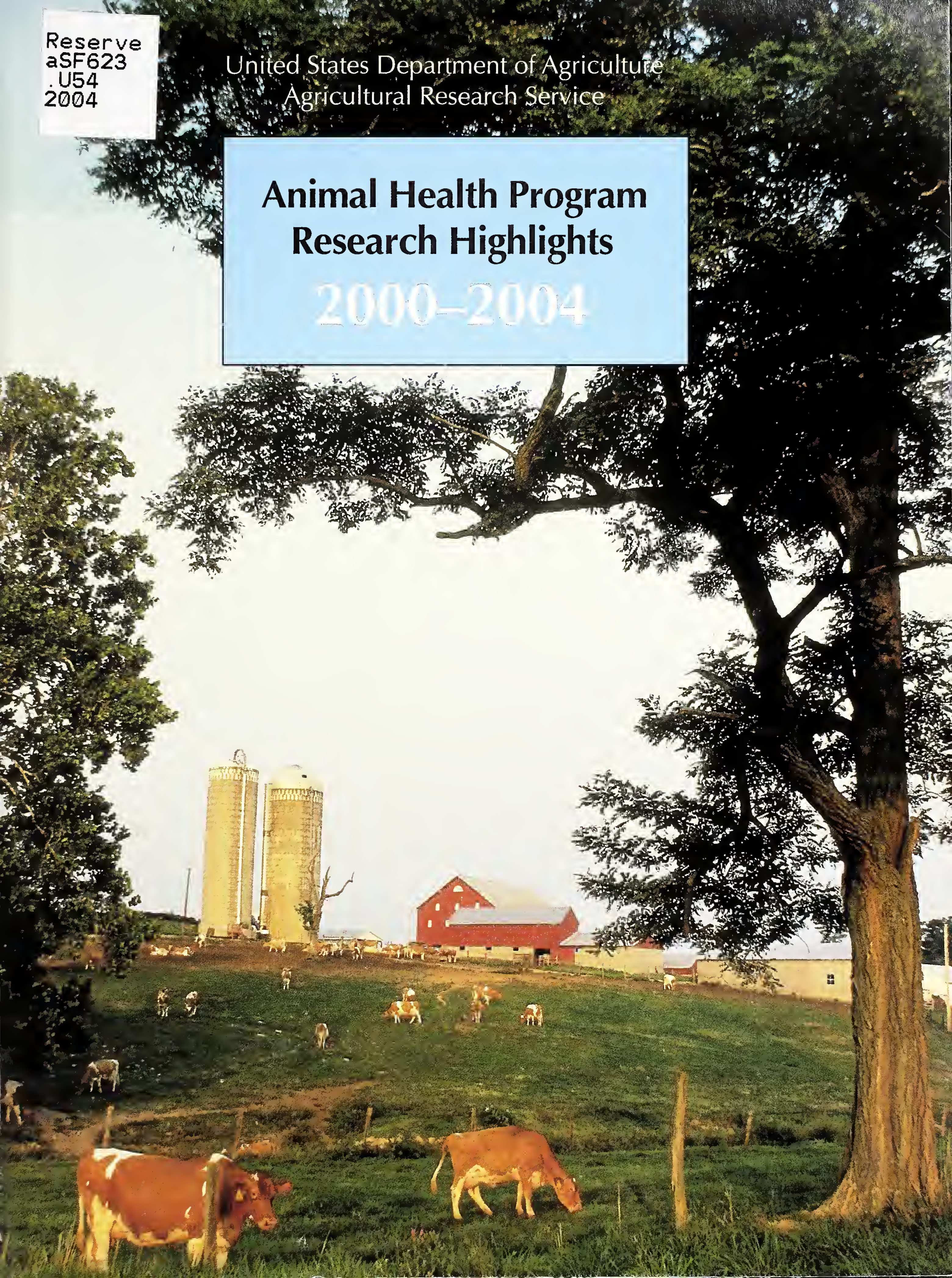
United States Department of Agriculture
Agricultural Research Service

Animal Health Program Research Highlights

2000–2004



Foreword

This *Agricultural Research* magazine reprint contains articles published from February 2000 to February 2004 addressing research conducted at various area research units and experimental stations as part of the U.S. Department of Agriculture, Agricultural Research Service's Animal Health Program.

The mission of the Animal Health Program is to conduct basic and applied research on selected diseases of economic importance to the U.S. livestock and poultry industries. The goals of the research mission are to produce knowledge and technology to reduce economic losses from infectious, genetic, and metabolic diseases of livestock and poultry.

The research components of this program include:

- Pathogen Detection and Diagnostics
- Animal Immunology
- Microbial Genomics
- Mechanism of Disease
- Genetic Resistance to Disease
- Epidemiology of Disease
- Strategies to Control Infectious and Non-Infectious Disease

Dr. Robert A. Heckert, National Program Leader, Animal Health, and Dr. Cyril G. Gay, National Program Leader, Animal Health and Safety, lead this ARS research program. Dr. Gay is lead on domestic disease issues and vaccine and drug discovery projects, and Dr. Heckert is lead on projects involving foreign and emerging diseases. The Animal Health National Program currently includes 60 research projects supported by 114 scientists located at 14 research sites throughout the country.

For additional information about the National Programs and the more than 1,200 research projects carried out by ARS, visit www.ars.usda.gov/research/programs.htm.

Robert Heckert
ARS National Program Leader
Animal Health
Beltsville, Maryland

Cyril Gay
ARS National Program Leader
Animal Health and Safety
Beltsville, Maryland

The articles in this volume are reprinted from *Agricultural Research* magazine, which is published by the U.S. Department of Agriculture, Agricultural Research Service, 5601 Sunnyside Ave., Beltsville, MD 20705-5130; phone (301) 504-1651; fax (301) 504-1641. The magazine is available on the World Wide Web at www.ars.usda.gov/is/AR/.

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Issued October 2004

Contents

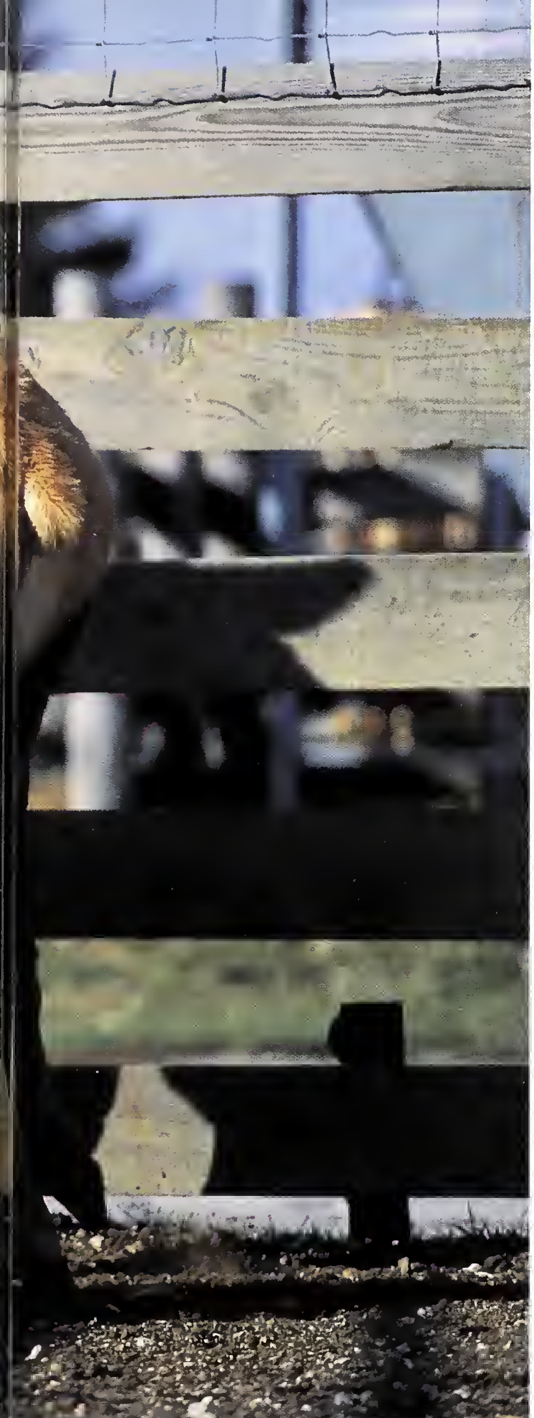
Cover: A small dairy farm in western Maryland. Photo by Scott Bauer. (K8502-1)

Zoonoses!

Bison and elk are the remaining reservoirs of brucellosis in the United States. RB51, the new vaccine that protects bison from the disease, will be evaluated for protection of elk (shown above).

KEITH WELLER (K8751-1)

Microbiologist Diana Whipple (left) and animal caretaker Katy Lies offer treats to a white-tailed deer being used to study tuberculosis in its wild counterparts.



KEITH WELLER (K8753-1)

Zoonoses—animal diseases that are naturally communicable to humans—have inflicted health problems on millions of people worldwide. But the power of three devastating zoonotic diseases—brucellosis, leptospirosis, and tuberculosis (TB)—may someday be broken up by new knowledge of how they are transmitted from wildlife to domestic animals to humans. Agricultural Research Service researchers at the National Animal Disease Center (NADC) in Ames, Iowa, are gaining this knowledge.

White-tailed deer in northeast Michigan have recently been identified as a wildlife reservoir of TB, which is caused by *Mycobacterium bovis*. The bison is a natural host for brucellosis. Leptospirosis, also called Weil's Disease, is transmitted to humans mainly through direct contact with infected animals, but it can also sicken humans via contaminated soil or water.

"Elk, deer, and bison threaten U.S. brucellosis and tuberculosis eradication efforts by presenting the opportunity for reinfection," according to ARS veterinarian Carole A. Bolin, leader of bacterial disease research.

As USDA's chief scientific research agency, ARS assists and advises other USDA agencies working with zoonotic diseases—the Animal and Plant Health Inspection Service (APHIS) and the Food Safety and Inspection Service (FSIS)—and other federal agencies like the Centers for Disease Control and Prevention (CDC) in Atlanta, Georgia.

TB Transmission

Milk is pasteurized to safeguard humans from becoming infected with *M. bovis*. The incidence of TB in cattle has declined steadily since 1917 when the state-federal eradication program was begun. In 1992, however, there was a resurgence of the disease. In addition, tuberculosis in captive deer and elk was recognized as a growing problem.

The presence of TB in wild white-tailed deer in Michigan poses a serious threat to the program to eradicate the disease from domestic livestock. ARS scientists have been conducting research on TB in white-tailed deer to determine how to control and one day eliminate it.

ARS microbiologist Diana Whipple and ARS veterinarian Mitchell V. Palmer developed a method to experimentally infect white-tailed deer with *M. bovis*. This method has been used to study the transmission of TB from experimentally infected deer to noninfected deer in research pens at NADC.

Their work has provided the first animal model to study TB transmission in white-tailed deer. "White-tailed deer experimentally infected with *M. bovis* developed lesions similar to those found in naturally infected deer," says Whipple.

In other studies, Whipple says NADC researchers have identified a possible route of transmission of *M. bovis* from experimentally infected deer to other animals. "We found *M. bovis* in deer saliva and nasal and tonsil secretions. Therefore a cow or another deer might become infected with *M. bovis* by eating feed contaminated with these secretions," says Whipple.

DNA fingerprints show that both wild and captive deer in Michigan are infected with the identical strain of *M. bovis* recovered from coyotes, raccoons, a bear, and cattle.

Tracking Leptospirosis

From Michigan deer to a public body of water in Springfield, Illinois, and in remote areas of Nicaragua, ARS researchers have tracked another bacterial disease that plagues animals and humans. Leptospirosis is caused by spiral-shaped bacteria called spirochetes (SPY-row-keets). Infected domestic animals and wildlife harbor these bacteria, more than 200 of which can cause leptospirosis.

To complicate matters, some animal species can be a host to several different bacterial strains, although usually animals are infected with only one type at a time. Humans can contract the disease, which is treatable with antibiotics, from urine if traces come in contact with the membranes around their eyes and mouth.

An international expert on zoonotic diseases, Carole Bolin traced the cause of a human outbreak of leptospirosis in Nicaragua to dogs. (See "Cracking the Hard Cases," *Agricultural Research*, June 1996, p. 4.)

In June 1998, Bolin was called by the CDC to help investigate the cause of a feverish illness in more than 100 U.S. athletes who became ill after swimming

in Lake Springfield. The illness resembled leptospirosis. NADC researchers tested water samples from the lake and isolates of bacteria from the patients. They also surveyed the livestock and wildlife residing near Lake Springfield. Laboratory tests confirmed the presence of pathogenic leptospires in the lake; however, the scientists were not able to identify the specific animal source.

New Vaccines

In cattle, leptospirosis causes abortions, stillbirths, and reproductive inefficiency. The NADC researchers have studied this disease since 1987. Their studies show that previous commercial vaccines for cattle have not adequately pro-tected them against some types of leptospirosis.

However, "a new vaccine, developed by BioCore in Omaha, Nebraska, gives 100 percent protection to cattle. Use of the vaccine blocks bacterial colonization in the urinary and reproductive tracts of the cattle," says Bolin. She and her research team are gathering data to support licensing of the commercial vaccine.

This is not the first time NADC researchers have supported and tested new vaccines to protect cattle against zoonotic diseases. ARS veterinarian Steven C. Olsen continues to explore the use of *Brucella abortus* strain RB51 in adult bison.

"Brucellosis in bison is very similar to brucellosis in cattle," says Olsen. He and the research team of Mark G. Stevens, Mitchell V. Palmer, Shirley M. Halling, Betsy J. Bricker, and Norman F. Cheville extensively tested RB51 for cattle. Because of their efforts, RB51 was approved by the USDA as the official vaccine to protect U.S. cattle against brucellosis, which costs U.S. beef and dairy producers nearly $30 million annually. This was the first time in over 50 years that a new vaccine was approved for brucellosis in cattle. RB51 replaced strain 19, a vaccine that is essentially no longer used.

Preliminary data suggest that the RB51 vaccine also protects bison against brucellosis. A larger study of bison heifer calves—now under way—should provide more conclusive data on the efficacy of the RB51 vaccine for calves. The ani-mals were vaccinated as calves and have been growing up. Once they get preg-nant, the bison will be challenged with a virulent strain of *B. abortus* to evaluate whether the RB51 vaccine protects them against abortion or infection. This study, begun in 1996, will not be concluded un-til the spring of 2000.

To lay the groundwork for commercial use of RB51 in bison, Olsen collaborated with scientists from the Wyoming Game and Fish Department and APHIS to evaluate the potential effect of the vaccine on several nontarget species. "RB51 did not cause visible signs of disease in birds, rodents, or other wild species. What we know about using RB51 in bison calves is that the young animals donÕt shed the vaccine strain; it persists longer in their lymph nodes than it does in adult cattle.

"By next spring, we may have enough data to obtain a conditional approval for using RB51 in bison calves," says Olsen. Since 1996, commercial use of RB51 has been only in calves—both bison and cattle. The results of inoculating bison calves with RB51 should pave the way for considering its use in a program to control bison brucellosis in Yellowstone National Park. This decision will be made by the National Park Service.—By **Linda McGraw,** ARS.

This research is part of Animal Health, an ARS National Program (#103) described on the World Wide Web at http://www.nps.ars.usda.gov/programs/appvs.htm.

All of the researchers mentioned in this article are at the USDA-ARS National Animal Disease Center, Bacterial Diseases of Livestock Research Unit, 2300 N. Dayton Rd., Ames, IA 50010; phone (515) 663-7325, fax (515) 663-7458, e-mail cbolin@nadc.ars.usda.gov dwhipple@nadc.ars.usda.gov solsen@nadc.ars.usda.gov. ◆

KEITH WELLER (K8750-1)

Veterinarian Carole Bolin prepares to inject a cow with the new vaccine for bovine leptospirosis.

A New, Rapid Test for Avian Influenza

Avian influenza is a vexing problem for poultry producers in the United States. One strain of the virus that causes the disease, H7N2, has been endemic in live-bird markets in the Northeast and Florida since 1994. These markets sell a broad variety of live poultry, often to specific ethnic markets. Consumers can choose either at-home or in-shop preparation of the bird.

Unfortunately, live-bird markets also serve as central mixing areas for avian influenza viruses and can harbor them for a long time. These markets can act as reservoirs from which viruses can potentially spread to larger, commercial facilities. Current regulatory efforts are ineffective in eradicating the virus.

ARS veterinary medical officer David Suarez has developed a test to quickly identify H7N2 presence in a flock. It's called an RRT-PCR test, short for real-time, reverse-transcription, polymerase chain reaction. Suarez's test, using a fluorescent probe, produces results in less than 3 hours.

Avian influenza infections can range from subclinical (with no symptoms), to mild (with production losses), to severe (with high rates of illness and death). The deadly form is called HPAI, short for highly pathogenic avian influenza.

"It can be difficult to identify the mild form because it is hard to differentiate it from other, more pedestrian health problems the flock exhibits," said Suarez, who is in the Poultry Disease Research Unit, in Athens, Georgia.

Suarez's test uses the virus's genetic code to identify it. The gene that identifies H7N2 avian influenza virus is the hemagglutinin gene. It's a rapidly evolving gene that has a high rate of amino acid substitutions, which may seem small in the grand scheme of the virus's genome. But each substitution moves the virus from a mildly pathogenic strain closer to a highly pathogenic strain—one that can kill an entire flock in as little as a week.

The last HPAI outbreak in the United States occurred in Pennsylvania in 1983 and 1984. Through combined federal, state, and industry efforts this outbreak was controlled. But milder avian influenza viruses were isolated from live-bird markets in several states from 1986 to 1989.

Recently, in Virginia, a mild form of avian influenza infected 197 flocks, and 4.5 million birds had to be killed to prevent further spread of the virus. Though the virus remained fairly innocuous, it had the potential to mutate and become deadly.

The mild-form H7N2 virus has been found in commercial poultry operations at least three times in the last 5 years, causing disease and serious economic losses for the industry.

"The costs of the 1983–84 outbreak were staggering: $63 million in federal funds and $350 million in increased consumer costs. This new test may avoid a replay of that devastating scenario by identifying the viruses earlier and with more accuracy," said Suarez.—By **Sharon Durham,** ARS.

This research is part of Animal Health, an ARS National Program (#103) described on the World Wide Web at http://www.ars.usda.gov.

David Suarez is in the USDA-ARS Poultry Disease Research Unit, Southeast Poultry Research Laboratory, 934 College Station Road, Athens, GA; phone (706) 546-3479, fax (706) 546-3161, e-mail dsuarez@seprl.usda.gov. ★

PEGGY GREB (K9808-1)

David Suarez, veterinary medical officer, and Suzanne DeBlois, biological science laboratory technician, prepare to load chicken tracheal swab samples into a real-time PCR machine.

Zapping Airborne *Salmonella* and Dust

Agricultural Research Service scientists have found a way to reduce *Salmonella* and dust in poultry areas. The technology may sound commonplace, but for many in the poultry industry it's exciting news.

The technology uses a negative electrostatic charge to remove dust from the air. Unlike most air cleaners, this device does not require air to move through it for cleaning to occur. Reducing the dust is important, because these particles often give hitchhiking germs a free ride into chicks' lungs and feathers.

Bailey W. Mitchell, an agricultural engineer, developed the ionizer system, in cooperation with veterinarian Henry D. Stone. Both researchers work at ARS' Southeast Poultry Research Laboratory in Athens, Georgia. ARS applied for a patent on the technology in July 1998. The first industry trials began in June of 1998, and a commercial product is now available.

Early trials in 1994 suggested the process would reduce dust and had the potential to reduce airborne transmission of Newcastle disease virus and other disease organisms such as *Salmonella*.

"When Bailey first started this work, we tested it in a small chick hatcher," says Stone. "He modified it many times. When I saw the consistent reduction in dust particles and bacteria during hatch, I knew it had potential."

Mitchell says credit is also due to veterinary medical officer Daniel J. King, physiologist R. Jeff Buhr, and microbiologists Peter S. Holt, Richard K. Gast, K.H. Seo, Mark E. Berrang, S. Stan Bailey, and Nelson A. Cox for their collaboration with this research.

Dust Spreads Disease

Keeping hatching cabinets free of pathogens is especially important, because one infected hatching chick can very quickly spread disease organisms to an entire cabinet of 15,000 tiny birds. One reason: The strong air needed to move warmth throughout the cabinet also moves dust.

Currently, chemical sprays are the only effective means of reducing airborne disease transmission in hatching cabinets, but they can be expensive and can damage hatching equipment.

This electrostatic system would be safer for poultry and other livestock. It would also keep dust levels down better than existing methods and would continually clean the air of pathogens.

KEN HAMMOND (K8649-1)

Agricultural engineer Bailey Mitchell demonstrates an electrostatic air cleaning system. The hatching cabinet used here is a small version of ones used commercially for hatching chicks.

The Simco Company of Hatfield, Pennsylvania, is one of the world's largest manufacturers of electrostatic equipment. Mitchell says the company provided electrostatic insights, equipment, and instrumentation under a federal-industry cooperative research and development agreement.

"It makes sense that reducing the fluff in the hatching cabinet would reduce bacterial contamination at pipping," says Hank Engster, vice president of technical service for Purdue Farms of Salisbury, Maryland. Pipping is when the chick breaks through its shell during hatching.

"We are pursuing a test of the technology at one of our complexes on the Delmarva Peninsula," says Engster.

Experiments conducted in a small chamber with agar plates exposed to a continuous *Salmonella* aerosol showed that high levels of charge can, on average, reduce airborne *Salmonella* levels from over 1,000 per plate to near 0 in what appears to be an instantaneous sterilizing effect.

The electrostatic technology consistently reduced *Salmonella* transmission between chicks by 98 percent and reduced *Salmonella* in air samples by 95 percent in a room with *Salmonella*-infected egg-laying hens.

In other tests, Mitchell built up hatching cabinet dust levels to 40 times above normal. The device reduced airborne particles by 99 percent in 60 seconds.

The system was tested on a hatching cabinet with a few infected fertile eggs interspersed among healthy ones. *Salmonella* counts in the guts of 7-day-old chicks in the cabinet with the device were reduced by a factor of 1,000- to 10,000-fold, when compared to counts in chicks in a hatching cabinet without the device.

Producers May Flock to Air Cleaners

"We are mainly interested in the technology for food safety—but also for improved growth and productivity in our flocks," says Purdue's Engster. "We sent a group down to Athens, Georgia, to assess how well the technology would meet our needs. Bailey showed us a system installed at Seaboard Farms."

Seaboard Farms in Athens supplies poultry for many fast-food companies. The company, with four hatcheries, produces over 5 million chicks a week.

Installing the ARS ionizer costs about $2,500 per hatching cabinet. Seaboard Farms hopes to install it in all of the cabinets in one hatchery.

"We tested the technology at our hatcheries," says Steve Bolden, vice president of live production at Seaboard Farms. "We found it reduced bacteria in three out of five tests and consistently kept dust levels down. We have negotiated with ARS to license the technology."

In addition, hatchability—the percentage of eggs that produce live chicks—increased as much as 2.7 percent in tests of the system, thanks to reduced pathogens, Mitchell says. "Multiply this seemingly modest increase by the millions of hatching eggs farmers sell and you can see the potential."

The technology has also interested turkey producers. Wampler Foods of Harrisonburg, Virginia, the seventh largest U.S. broiler chicken producer and third largest turkey producer, invited Mitchell to demonstrate the technology. Wampler is interested and would like to install units when commercially available, according to Tom Knapp, manager of Wampler's turkey breeding operations. He says the company is also planning on model modifications to better fit their hatching cabinets.

"Initial tests in poultry production look promising in terms of improved vitality and health of flocks," says Knapp. "If we can verify reduced levels of bacteria, we think the technology would be a vital component to our overall live production health programs."

According to Mitchell, numerous simple ionizer systems have been developed and marketed for air-cleaning applications with little or no research. Although many of these devices had potential in small spaces with light dust loads, they require air to pass through them and are not able to handle the larger space and higher dust levels of a typical hatching cabinet. The supercharged ionizer/dust collection system developed by ARS appears able to do the job.

The process is likely to have applications outside agriculture, Mitchell says. In tests, the researcher removed smoke from a 3,300-cubic-foot room with 95-percent efficiency. Many other companies, he adds, are asking to review the technology for environmental and other air-cleaning applications.—By **Jill Lee,** formerly with ARS.

This research is part of Animal Health, an ARS National Program (#103) described on the World Wide Web at http://www.nps.ars.usda.gov/programs/appvs.htm.

Bailey W. Mitchell and Henry D. Stone are with the USDA-ARS Southeast Poultry Research Laboratory, 934 College Station Rd., Athens, GA 30605; phone (706) 546-3443 [Mitchell], (706) 546-3431 [Stone], fax (706) 546-3161, e-mail baileym@bae.uga.edu. ◆

KEN HAMMOND (K8649-2)

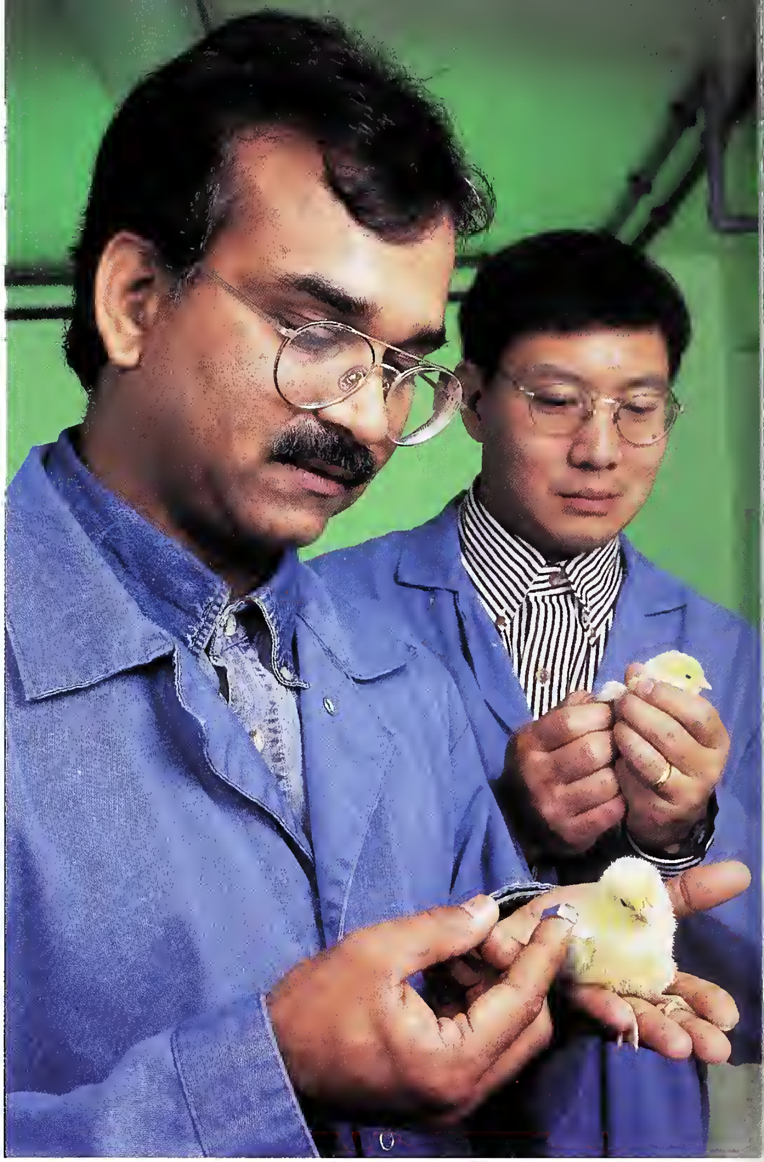
The petri dishes below show sterilization effects of negative air ionization on a chamber aerosolized with *Salmonella enteritidis*. The left sample is untreated; the right, treated.

Mapping
the Way to
Disease-Free Chickens

"The poultry industry is afraid it may start losing the vaccine race against Marek's disease, as ever more virulent strains appear and cause unbearable losses."
—**Hans Cheng**

The newest version of a chicken genome map gives Hans H. Cheng hope for developing a chicken resistant to Marek's disease, a viral disease that causes tumors in the birds.

PEGGY GREB (K8762-1)



▲ **Farm manager Raj Kulkarni (left) and geneticist Hans Cheng examine a day-old chick for disease resistance and susceptibility. Each chick is tagged with a wing band for identification.**

"The poultry industry is afraid it may start losing the vaccine race against Marek's disease, as ever more virulent strains appear and cause unbearable losses," says Cheng, a geneticist with USDA's Agricultural Research Service. "That's why using a genome map as a guide or road map to breeding chickens resistant to Marek's disease is a priority."

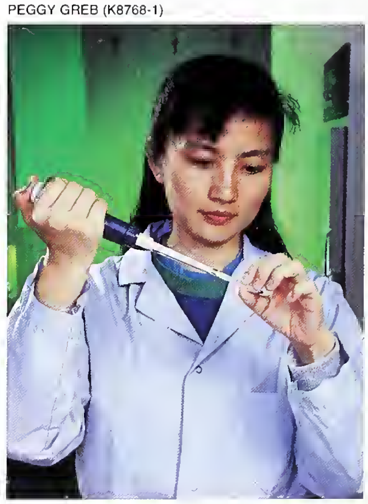
Before the first vaccine was developed in the late 1960s by scientists at the ARS Avian Disease and Oncology Laboratory in East Lansing, Michigan, the disease caused losses of $300 million a year. Those losses came from a combination of deaths, fewer eggs, and condemnation of carcasses at poultry slaughter plants. Even with the vaccine, losses can still run as high as $100 million a year.

The vaccine has to be updated periodically, in a race to keep ahead of ever more virulent strains. Cheng is counting on the genome map to help win the race.

"Chickens bred to resist Marek's would be the first generation bred with modern molecular techniques," Cheng says.

"The genome map will also help us build a superchicken, by helping us find the best combination of genes and proteins for resistance to many diseases as well as for productivity," Cheng

PEGGY GREB (K8768-1)



▼ **Research associate Hsiao-Ching Liu prepares a sample of chicken RNA. The samples are then run on DNA microarrays to screen thousands of genes simultaneously. This new technology is especially promising and should lead to the rapid identification of agriculturally important genes.**

Cheng says that mapping a genome is like mapping a city neighborhood. "First you need to use street signs as markers, then you go looking for individual houses or genes."

says. Although their focus is on Marek's first and then other diseases, Cheng and his colleagues are also searching for genes that will promote better and more efficient growth.

The latest map can be viewed on the WWW at http://poultry.mph.msu.edu/resources/conmap/conmap.htm. It is actually a composite of three maps, including one jointly constructed by the Avian Disease and Oncology Laboratory and its neighbor, Michigan State University in East Lansing. The other two maps come from the Compton Institute for Animal Health in England and the Wageningen Agricultural University in the Netherlands. The new map is the product of the International Chicken Genome Mapping Project begun in 1994.

Overlapping Maps

"This is the first such international effort," Cheng says, although individual countries such as the United States have worked on mapping chicken genes since 1936. "Chickens were the first farm animal to have their genes mapped. But, in the beginning, mapping was based on visible physical characteristics such as feather color, rather than today's biotechnology that allows DNA and RNA analysis."

PEGGY GREB (K8763-1)

Cheng, one of the co-coordinators of the East Lansing map project, along with Jerry B. Dodgson, a microbiologist at Michigan State, says the DNA samples used in making these maps come from the East Lansing lab and the Compton Institute.

The East Lansing lab sends these DNA samples around the world; they were taken from the blood of 52 chicks that were specially bred in 1990. The Compton Institute likewise sends vials of DNA samples around the world that are taken from a similar "reference family" of chicks. The samples were collected years ago and only from those individual chicks.

"So," Cheng says, "the DNA samples are in limited supply. But modern molecular techniques have greatly reduced the amount of DNA needed for mapping, so there no longer seems to be a danger of running out of samples."

All three maps used for the latest composite are genetic maps. The Compton Institute published the first such map. Dodgson is a few years away from a more detailed genome map. It is called a physical map because the breakpoints used to map genes are produced by a physical cutting of DNA fragments from chromosomes. This contrasts with the genetic map in which the breakpoints occur naturally, as a result of sexual reproduction. A physical map fine-tunes a genetic map, giving a higher resolution—like a more detailed street map.

Cheng says that mapping a genome is like mapping a city neighborhood. "First you need to use street signs as markers, then you go looking for individual houses or genes," he says.

"We have about 2,000 genetic markers to help us locate genes," says Cheng. "For chickens, somewhere between 2,000 and 4,000 genetic markers is a reasonable goal to begin to construct a genome map and locate genes. The problem is that about half of these markers have limited utility because they can only map an individual chicken's genome and that of its progeny. Unlike the rest of our markers, these markers don't always mark the same gene in the same spot for all other chickens," he says.

When Dodgson's physical map is ready, it or a composite version will be integrated with the composite genetic map. Overlapping the maps helps build a better genome map.

"Every time one researcher finds another marker, another street sign is found for the maps," Cheng says. Different maps are lined up to provide guides for where to go next to complete the map. A physical map may be deficient in markers so we can use a genetic map to find those markers and vice versa."

All Creatures, Great and Small, Share Some Identical Genes

The maps also benefit from being overlaid with those of the human genome and other animal species.

"It's surprising how well the human and avian genomes line up," Cheng says. The human genome and chicken genome projects complement each other. By lining up the two maps, human immunologists and avian health researchers can help locate genes for traits that improve disease resistance in both species.

"The amazing thing about evolution is that it leaves many species—from yeast to mammals—sharing some of the same large chunks of DNA," Cheng says. "The same mapping

techniques work in all species—plants and animals—with nuances caused by differences in biology and reproduction, for example."

Since tumors are so common in chickens, the first cancer-causing genes were isolated from chicken tumor tissue. A gene that causes cancer in chickens will have similarities to a gene that causes cancer in humans. The East Lansing avian lab contributed greatly to the work on human cancer in the 1970s.

The Ultimate Science

Collaboration among geneticists—sharing and comparing of genetic maps—is typical of how scientists often work together to discover something, Cheng says. And collaboration is particularly needed in genome mapping.

"In that sense, genome mapping is the ultimate science. We're all forced to collaborate, and we benefit from others' work," he says.

"The final step, after the maps are made and all the genes are sequenced, is to identify genes that influence the trait you're looking for—in this case resistance to Marek's disease," says Cheng.

As a practical matter, Cheng and his colleagues in effect work on all these steps somewhat simultaneously. They are drawing the map at the same time as they are driving city streets and looking for house addresses.

"We take the maps we have and use them to sequence and identify genes with resistance to Marek's," he says. He recently began a new DNA technique called microarray to find these genes. "It should pare years off the search," he says.

The microarray technique allows a search for a great number of genes at one time, rather than for gene markers, Cheng says.

PEGGY GREB (K8766-1)

Technician Laurie Molitor (left) and research associate Christiane Hansen analyze chicken genetic markers using DNA sequencers. These semiautomatic machines increase the number of samples that can be processed per day and minimize human errors.

"RNA is put on two microscope slides. The genetic material on one slide might be from a disease-resistant chicken, with the other slide containing RNA from a susceptible one," he explains.

"A quick check of such samples, en masse shows differing responses in RNA levels between the two. The differences show which genes may be responsible for the trait. We hope this new technology, combined with gene mapping, will enable the rapid identification of genes for disease resistance," he says.

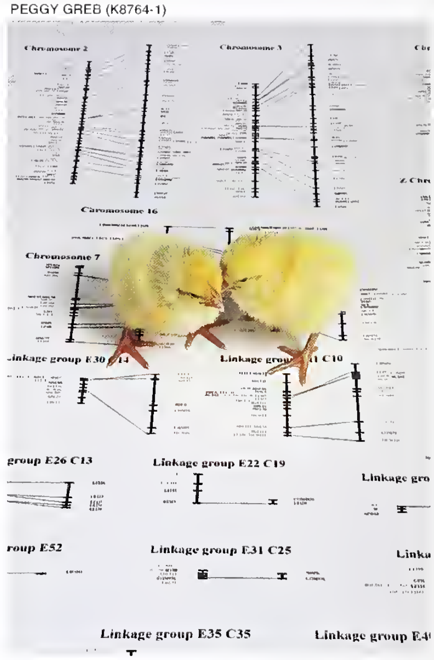
As another aid to gene identification, Cheng and his colleagues have also recently developed 19 inbred lines of chickens that have disease-resistance traits linked to one or a few genes, rather than to a complex of numerous genes. This makes identifying genes for disease resistance easier, and facilitates creating chickens that either are or are not disease resistant—nothing in between that would hamper the search, he says.

"This unique genetic resource will work for other traits as well, giving us the opportunity to quickly isolate the responsible genes," Cheng adds.—By **Don Comis,** ARS.

This research is part of Animal Genomes, Germplasm, Reproduction, and Development, an ARS National Program (#101) described on the World Wide Web at http://www.nps.ars.usda.gov/programs/appvs.htm.

Hans H. Cheng is with the USDA-ARS Avian Disease and Oncology Laboratory, 3606 East Mount Hope Rd., East Lansing, MI 48823-5338; phone (517) 337-6828, fax (517) 337-6776, e-mail hcheng@pilot.msu.edu. ◆

PEGGY GREB (K8764-1)



Chicks atop a picture of a genetic map of a chicken. The chicken genome has 39 pairs of chromosomes, whereas the human genome contains 23 pairs.

Getting to the Heart of Chicken Ailments

Humans aren't the only ones who are susceptible to congestive heart failure—the heart's inability to maintain adequate blood flow to tissues. Chickens, particularly broilers, fall prey to a similar condition called ascites.

In chickens, the right ventricle of the heart enlarges and can't pump blood efficiently to the lungs. Blood pressure then builds in the liver, and a yellow serumlike fluid leaks from the liver into the body cavity, eventually leading to death.

KEITH WELLER (K3627-16)

Baby chicks are being bred for resistance to ascites, a fatal disease resulting in heart failure in chickens.

The problem has been around for about 20 years in birds grown at high altitudes. But in the last decade, it has become a problem everywhere.

"Birds are genetically selected for fast growth. It now takes less than 6 weeks for birds to get to market," says poultry physiologist Janice M. Balog. "Their hearts and lungs have to work harder to keep up with the rapid rate of growth, and they just can't do it."

"Many factors, such as ammonia, dust, or respiratory diseases—combined with accelerated growth rates—have led to a growing problem among poultry producers," she says.

Balog, who is in the ARS Poultry Production and Products Safety Research Unit in Fayetteville, Arkansas, has made recent strides to prevent this fatal condition in poultry.

In an 8- by 12-foot room that holds 480 chickens, Balog's birds are taking a trip to higher elevations. This room, called a hypobaric chamber, simulates conditions found at higher altitudes. At a simulated 9,500 feet above sea level, 80 to 90 percent of commercial broilers will develop ascites.

Using the hypobaric chamber allows Balog to identify and selectively breed resistant birds and to test for possible remedies. In the fourth year of her study, Balog and University of Arkansas poultry geneticist Nicholas Anthony, have selected over four generations of broilers that have escaped this disease. One population exhibits no more than 20 percent ascites at simulated high altitudes.

"Once we are satisfied with the selected populations, we will attempt to determine what's different physiologically between the two lines," says Balog. "Ultimately, we hope to eliminate the disease."

Ascites research is particularly important because some birds die as early as 3 weeks old, and more die before making it to processing—after the producer has wasted a lot of money on feed costs that are passed on to the consumer. Finding new ways to prevent this disease will reduce the amount of money spent on birds that never make it to market.

Ascites research is particularly important because some birds die as early as 3 weeks old and more die before making it to processing.

Aside from genetic selection, Balog has found other ways poultry producers can reduce the incidence of ascites, including increasing ventilation in poultry houses and maintaining stable temperatures. Currently, producers restrict the amount of feed—a process that slows growth and reduces mortality—but birds on restricted diets take longer to reach market weight and can have less white meat—the most valuable part of the chicken.

"We're looking at restricting feed during certain time periods. This seems to help the problem," says Balog.—By **Tara Weaver-Missick,** ARS.

This research is part of Animal Health, an ARS National Program (#103) described on the World Wide Web at http://www.nps.ars.usda.gov/programs/appvs.htm.

Janice M. Balog is in the USDA-ARS Poultry Production and Products Safety Research Unit, University of Arkansas, Poultry Science Center, Room 0-303, Fayetteville, AR 72701; phone (501) 575-6299, fax (501) 575-4202, e-mail jbalog@comp.uark.edu. ◆

Infectious **Emergencies**

Creating the Tools To Study Immune Response in Farm Animals

A multivehicle accident just happened on U.S. Highway 100. Several witnesses grab their cell phones and dial 911. The dispatcher on the other end radios the nearest highway patrol cars and alerts fire and rescue squads to rush to the site. The first troopers on the scene assess the damage and radio back for more patrol cars and emergency personnel, while rescue workers use walkie-talkies to coordinate treatment of the injured.

Good communication turns chaos into coordination.

If, like Alice in Wonderland, you could shrink up and scramble down the digestive or respiratory tract of a farm animal, you could witness emergency scenes like this all along the way as viruses, bacteria, parasites—even worms—try to penetrate the lining of the animal's gut.

But instead of using radios and walkie-talkies, cells of the immune system use proteins called cytokines to orchestrate a response. You may have heard of some of them—the interferons and interleukins, for example. Others, like chemokines, are less well-known.

Over the last decade, cytokines have become a hot area of interest in the search for alternatives to antibiotics and other drug therapies for our food animals, says ARS immunologist Joan K. Lunney. She heads the Immunology and Disease Resistance Laboratory, at Beltsville, Maryland.

The laboratory is at the forefront of using cytokines to answer basic questions about the immune response of pigs and cattle. In pigs, for example, Lunney wants to know at what age the immune system starts functioning and how well it functions. The trick is to find which cytokines generate a protective immune response and which either distract the immune system or cause an over-response.

"We know many factors affect the quality and quantity of cytokine response to an antigen or infection," says ARS microbiologist Joseph F. Urban, Jr. Some responses are appropriate and protect the animal; others are inappropriate and further stress the animal.

"It's a balancing act—the yin-yang hypothesis of regulation."

Knowing which cytokines are key to maintaining this balance will enable scientists to design therapies that stimulate the desired response or suppress the undesired. Moreover, scientists will be able to select animals that have the genetic background for appropriate responses.

Reagents to the Rescue

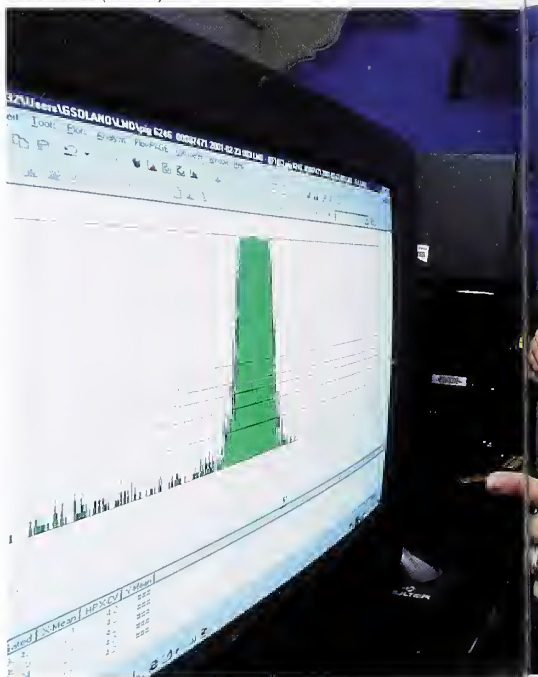
To look for changes in cytokine levels, one needs reagents. So Lunney's laboratory has become a kind of reagent factory, preparing tiny molecules that can be used to measure either the cytokines or changes in their production.

To measure a cytokine directly, the researchers make monoclonal antibodies—molecules that attach to specific sites on a particular cytokine. Using these monoclonals, they can measure the amounts of cytokines produced at each stage of an infection or a vaccine trial. With a sensitive fluorescence assay, they can even identify the exact cell making the cytokine.

To measure cytokine production indirectly, the researchers make "DNA competitors," which enable them to detect changes in expression of the gene that codes for the cytokine. When a cytokine isn't needed, levels of the gene product—messenger RNA—are low. When the gene gets turned on, messenger RNA levels rise.

Using the well-known PCR—polymerase chain reaction—the researchers make millions of DNA copies of

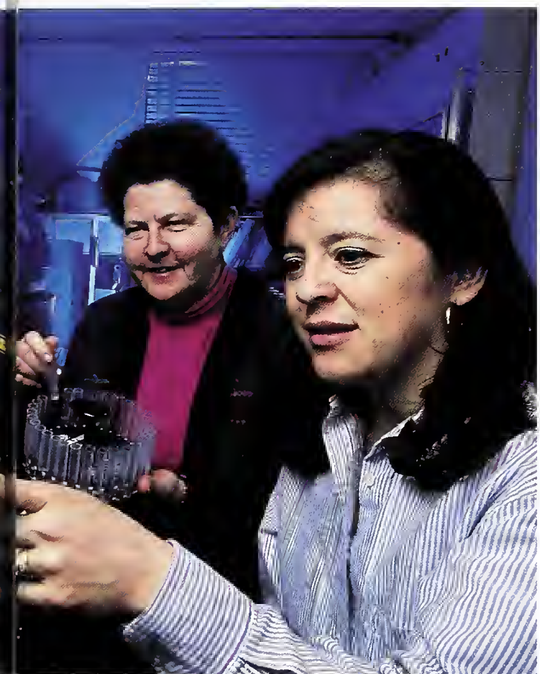
PEGGY GREB (K9477-1)



Research associate Gloria Solano-Aguilar (right) discusses data with immunologist Joan Lunney after stained cells were run and analyzed with a laser-based flow cytometer.

PEGGY GREB (K9474-1)

Molecular biologist Dante Zarlenga examines autoradiographic data to confirm the proper construction of reagents for studying changes in bovine and swine cytokine transcription.



PEGGY GREB (K9478-1)

Microbiologist Joseph Urban initiates a subclinical oral infection of young pigs with a gastrointestinal parasite to stimulate a strong cytokine response at mucosal surfaces in the gut. Support scientist Ethiopia Beshah prepares to rapidly process tissues from infected animals for cytokine gene expression.

messenger RNA for a given cytokine. Then they use PCR again to snip a chunk of bases—about 100 or so—out of the DNA copy. This yields a deleted version, called a DNA competitor. To measure gene expression for a specific cytokine, they use a known amount of its DNA competitor to serve as a kind of internal standard in the PCR assay.

So far, laboratory personnel have produced DNA competitors for 11 cytokines in pigs and 16 in cattle. The researchers draw from the large database of human and mouse genes to prepare their reagents.

"We have sent DNA competitors for swine and cattle to hundreds of investigators around the world," says ARS microbiologist Dante S. Zarlenga. He works with cattle and swine cytokines and developed a simple technique for making DNA competitors.

His colleague, microbiologist Louis C. Gasbarre, has used the reagents to develop a framework of cattle immunity that researchers worldwide can build on.

It's Beyond Complex

Researchers at Lunney's laboratory are working to get the big picture of immune response in cattle and pigs. And this picture is more than complex. It will differ depending on whether the infectious agent is a virus, bacterium, protozoan parasite, or worm, whether it's a combination of these agents, what tissue is infected, and how long the infection has been around.

Zarlenga is working on what he calls third-generation assays from farm animals, which will show changes in expression of many cytokines at once. He says the technology already exists to "print" thousands of minuscule dots of functional DNA on a single microscope slide.

Like magnets, these dots will attract their complementary DNA—the copies researchers make from the cells they are studying. By adding fluorescent labels to the complementary DNA, researchers can estimate the degree of cytokine expression by the brightness of each dot.

The technology is up and running for human and mouse DNA, says Zarlenga. He and Gasbarre are producing cytokine DNA from cattle for each of the spots on the slide.

Meanwhile, Lunney collaborated with researchers at the University of Illinois-Urbana and Pharmacia Animal Health in Kalamazoo, Michigan, to produce monoclonal antibody panels for two pig cytokines. She says each antibody in a panel attaches to a different site on the cytokine, allowing accurate measurement. Trouble is, panels exist for less than one-third of the pig cytokines and are only slowly becoming available.

"Most people want to measure the protein," Lunney notes, "because you can actually determine which immune cell is making it." Eventually, she hopes there will be antibody arrays for pigs and cattle like the DNA arrays Zarlenga is working on.—By **Judy McBride,** ARS.

This research is part of Animal Health, an ARS National Program (#103) described on the World Wide Web at http://www.nps.ars.usda.gov.

Joan K. Lunney, Dante S. Zarlenga, Louis C. Gasbarre, and Joseph F. Urban, Jr., are with the USDA-ARS Immunology and Disease Resistance Laboratory, 10300 Baltimore Ave., Bldg. 1040, Beltsville, MD 20705-2350; phone (301) 504-8201, fax (301) 504-5306, e-mail jlunney@anri.barc.usda.gov jurban@anri.barc.usda.gov zarlenga@anri.barc.usda.gov lgasbarr@anri.barc.usda.gov. ◆

Cracking the Code of Marek's Disease

In an advance that could usher in new vaccines for protecting poultry, Agricultural Research Service (ARS) scientists have sequenced the genetic code of the chicken herpesvirus that causes Marek's disease.

Worldwide losses attributed to Marek's are an estimated $1 billion annually. The disease manifests as tumors on the bird's spleen, liver, lung, kidney, and other tissues. Other disease symptoms include neurological disorders, such as partial paralysis in the bird's legs or wings.

Since the 1960s, poultry producers have used vaccines made from benign or "crippled" Marek's strains to immunize birds. But the virus has countered each new vaccine with ever more virulent strains, once again imperiling the industry.

Now, with the virus's genetic code in hand, ARS researchers have begun studying the molecular mechanisms by which it causes disease. "Our aim is to create new vaccines through genetic engineering to protect poultry against hot [virulent] strains of Marek's disease viruses," says Sanjay M. Reddy, a medical veterinary officer at ARS' Avian Disease and Oncology Laboratory, in East Lansing, Michigan.

Reddy's colleagues there are Robert F. Silva, Richard L. Witter, and Lucy F. Lee, who decoded the Marek's strain called MDV1-GA.

Another ARS team, led by Daniel L. Rock and Gerald F. Kutish at the agency's Plum Island Animal Disease Center in New York, sequenced another MDV1 strain, described as Md5 vv (very virulent), and a harmless variant in turkeys called sterotype 3, which is used to vaccinate chickens.

Mapping a Plan of Attack

Using technology called a high-throughput sequencer, Rock's group decoded the order of 177,874 pairs of chemical "letters," called nucleotides, comprising the Md5 vv strain's DNA alphabet for 104 genes.

"Having a pathogen's genetic blueprint is just like having the opposing team's playbook with its strategies for success; if you know what the game plan is, you can find ways to interfere with it," says Rock, who leads the center's African Swine Fever Research Unit.

The advance could also give rise to new diagnostic tools for predicting where and when new strains emerge in the field and how virulent they are, says Rock.

Located off Long Island, New York, the Plum Island lab's prime objective is decoding the genomics of exotic animal pathogens for diagnostics and vaccine development. Sequencing the two poultry viruses was a special assignment facilitated by the lab's high-throughput DNA sequencer and expertise in viral genomics.

Using a different method, Lee's team charted a genetic stretch of MDV1-GA called the "unique long region," encompassing 113,508 nucleotide letters. "GA is a prototype of MDV that scientists all over the world have been working on for

KATHY APICELLI (K9493-1)

At the Plum Island Animal Disease Center, microbiologists Edan Tulman (left) and Claudio Afonso perform high-throughput capillary DNA sequencing, a technique that enables rapid determination of the complete genetic content of a viral pathogen.

K9494-1

Normal chicken eye on left. Eye lesions and irregular pupil caused by Marek's disease on right.

over 20 years," notes Lee. Both GA and Md5 strains of MDV1 are oncogenic (cancer-causing), and their sequences will reveal genes unique only to this group, she adds.

Lee's colleague, ARS molecular biologist Silva, is now comparing the blueprints of these viruses with other herpesviruses. Such comparative studies could also yield more information about the virus's biological functions and could reveal specific genes that make particular MDV1 strains more virulent than others.

Cellular Saboteur

The vaccines work by presenting the bird's immune system with an antigen, normally a protein, from a benign or crippled virus. This primes the bird's system to custom-make "killer cells" and antibodies that will mobilize specific attacks against virulent MDV1 strains.

"You do need more than just vaccines," Witter acknowledges. "You need good management and good, resistant bird stocks. But without a vaccine, you'd be dead in the water. It can make the difference between 20 to 60 percent of birds dying in a layer flock, or not."

Charles Beard, vice president of research and technical programs at the U.S. Poultry and Egg Association in Tucker, Georgia, agrees: "For poultry, that's 99.9 percent of the hope we have" against Marek's.

Even with vaccines, the disease still costs the U.S. poultry industry millions of dollars annually in losses from bird deaths, diminished egg laying, and carcass condemnations at processing plants.

Catch Me If You Can

The emergence of new strains—such as Md5 vv—coupled with today's high-density poultry production, only exacerbates the problem. Beard puts it this way: "Over the years, these strains out in the field have gotten hotter and hotter, and we've run out of bullets."

"It's been a cat-and-mouse game over the past 2 decades," adds Reddy. That's roughly when some of the earliest chicken vaccines began losing their effectiveness to more virulent MDV1 strains.

Historically, scientists have produced the chicken vaccines by selecting strains either from field samples or lab research that, through natural mutations, replicate inside their host without causing serious harm.

But finding suitable vaccine candidates can be a time-consuming, hit-or-miss affair. Witter notes, "Grow a virus long enough in an artificial system and it will mutate. If you catch them at the right time, these mutations can become useful in creating new vaccines."

Now, with the letters of the virus's DNA alphabet spelled out, he adds, scientists can begin pinpointing specific genes of interest, such as those responsible for causing tumors in chickens.

"It's like having a road map with street signs; you know exactly where you're going," says Lee, who works with Reddy and Silva on the recombinant vaccines.

Cut and Paste

One way scientists learn what the genes do is to delete, or "snip," them from the virus's DNA using enzymes. They then inoculate chickens with the genetically altered virus. This shows whether it will replicate, cause disease symptoms, or preferably stimulate an immune-system response.

The Avian Disease and Oncology Laboratory has already applied the technique to study some of the genes involved in oncogenesis. "Once you understand how these genes work, you can design recombinant vaccines that protect against very virulent strains of MDV1," says Silva.

Recombinant vaccines that show promise will be compared with commercial vaccines, he adds. If they still measure up, the lab will transfer the technology to a private company for further research and development.

Even then, biotechnology's best chicken vaccine may not arm poultry farmers with the proverbial magic bullet. "You'd have to come up with a strategy that not only stops the virus from causing disease, but also keeps it from evolving so that it won't beat you again," Reddy ventures.

Maybe the clue lies waiting, hidden within Marek's genetic code.—By **Jan Suszkiw,** ARS.

This research is part of Animal Health, an ARS National Program (#103) described on the World Wide Web at http://www.nps.ars.usda.gov.

To reach scientists mentioned in this article, contact Jan Suszkiw, USDA-ARS Information Staff, 5601 Sunnyside Ave., Beltsville, MD 20705; phone (301) 504-1630, fax (301) 504-1641, e-mail jsuszkiw@ars.usda.gov. ◆

Battling Bisons' Mysterious MCF Disease

JACK DYKINGA (K5680-1)

A disease that has buffaloed scientists, veterinarians, and bison ranchers is yielding some of its secrets. That's because of innovative research by ARS scientists and their Washington State University colleagues. They've developed new tests for detecting and correctly identifying the disease, known as malignant catarrhal fever, or MCF. These tests are the work of veterinary microbiologist Hong Li of the ARS Animal Disease Research Unit and veterinary virologist Tim B. Crawford of Washington State University, both in Pullman, Washington.

"MCF's history in veterinary medicine can be traced back to the late 1700s," Li says. "The disease affects many domestic and wild ruminants—animals that have multichambered stomachs—such as cattle, bison, and deer. It is caused by a group of herpesviruses with very complex life cycles. Several ruminants serve as carriers of MCF viruses. In the United States, the most prominent carrier is the domestic sheep."

Sheep Don't Succumb

"Sheep MCF virus, or ovine herpesvirus 2, is the cause of most MCF cases in the United States," says Li. "Sheep carry MCF virus, but apparently are not susceptible to the disease. It is often fatal to some other animals but is harmless to humans.

"MCF occurs sporadically," adds Li. "There's much about it that we still don't understand. Though both cattle and bison can die from the disease, bison seem more susceptible. In fact, MCF is one of the leading infectious diseases of bison, so it's a top research priority of the American bison industry."

Some 300,000 bison are currently being raised in the United States for their unique, low-fat meat. That's according to Donal O'Toole, who collaborates with Li and Crawford. O'Toole is a veterinary pathologist at the University of Wyoming, Laramie.

Sheep inadvertently spread the virus, mainly from their nasal secretions. Bison or other animals sharing the same range, pasture, feed or, perhaps, water, with the sheep may come into contact with the virus particles shed by the sheep. Notes O'Toole, "The virus doesn't live very long once it's shed."

An early and telltale sign of the disease is a severe runny nose and often a custardlike discharge that eventually encrusts the afflicted animal's muzzle. Other symptoms that follow may include mouth ulcers; cloudy, whitened eyes; swollen lymph nodes; bloody diarrhea; and a high fever—as much as 107°F, as compared to a healthy bison's normal 101° to 102°F.

Tests Identify Antibodies and Virus

Today there is no treatment or cure for MCF and no vaccine. Yet the tests that Li and Crawford developed may someday help prevent this disease.

One assay is a CI-ELISA, short for competitive inhibition enzyme-linked immunosorbent assay. This blood test is best used for screening healthy herds of MCF-susceptible animals—bison or cattle, for example. It determines whether any of the animals, even if they are not showing any signs of illness, are carrying an MCF virus.

Li points out, "Besides screening MCF-susceptible animals, the test is also very useful for screening carrier animals, such as sheep, for MCF virus." The team's CI-ELISA can detect even very small amounts of antibodies that the animal makes in response to the invading virus. Li explains that the CI-ELISA is the first test capable of detecting antibodies that are formed in response to MCF viruses. It is a significant improvement over earlier MCF blood tests.

PEGGY GREB (K9876-1)

Veterinary microbiologist Hong Li (left) and veterinary virologist Tim Crawford select sheep for an MCF transmission study.

Although the assay can indicate whether an animal has made antibodies to MCF, it can't distinguish among members of the MCF virus family. That's the job of tests that are based on what's called a polymerase chain reaction, or PCR.

"Researchers in Scotland," says Li, "developed a PCR for sheep MCF virus. We adapted that to develop other PCRs, including a quantitative one for MCF research. The quantitative PCR tells us not only which MCF virus is present, but also how much of it there is."

PCR tests are useful for identifying new MCF viruses. For example, Li's group was the first to identify a new MCF virus in domestic goats. What's more, they used PCR technology to discover another new MCF virus that causes the disease in white-tailed deer. Li did the deer work with veterinary pathologist Neil W. Dyer at North Dakota State University.

Having this array of PCRs may help reveal which viruses in the MCF family are deadly to which species of livestock or wildlife. That information could help livestock producers, wildlife specialists, and zoo managers.

Diagnostic Tests Prove Useful

Today these tests can be performed at regional veterinary diagnostic laboratories on behalf of researchers and veterinarians who send in specimens. For instance, the Washington Animal Disease Diagnostic Laboratory at Washington State University processes between 800 and 1,000 MCF diagnostic assays a year in addition to the thousands it runs for research purposes.

Zoos are also increasingly relying on the tests, reports Li. "They want to test their own animals as well as those they are interested in adding to their collections."

Li and Crawford have used the assays to determine a previously unknown interval during which newborn lambs are virus-free. "We found that most lambs are virus-free for about 6 to 8 weeks after birth," Li states. "So you can establish a virus-free flock if you take lambs from their infected mothers before that time is up."

For that work, they collaborated with ARS animal scientist Gary D. Snowder at the agency's U.S. Sheep Experiment Station, Dubois, Idaho. Li says several zoos in North America have begun using this regimen to produce MCF-free sheep.

In addition, the tests have been "very crucial to bison MCF research," asserts Li. Says colleague O'Toole, "The tests, for example, have shown that cattle are significantly less susceptible to ovine herpesvirus 2 than are buffalo."

A study by researchers in Pullman and Laramie revealed that at least 25 to 35 percent of all bison are infected with the MCF virus.

Li received a top regional award from ARS in 2000 for his pioneering research. In addition, he has served as special expert for the United Nations in establishing diagnostic assays for MCF in West Africa.

PEGGY GREB (K9875-1)

To test for antibodies to MCF virus, technicians Jan Keller (back) and Lori Fuller conduct a CI-ELISA.

Li and colleagues have reported their findings in the *Journal of Clinical Microbiology* and the *Journal of General Virology* as well as many veterinary science journals, including the *Journal of Veterinary Diagnostic Investigation.*

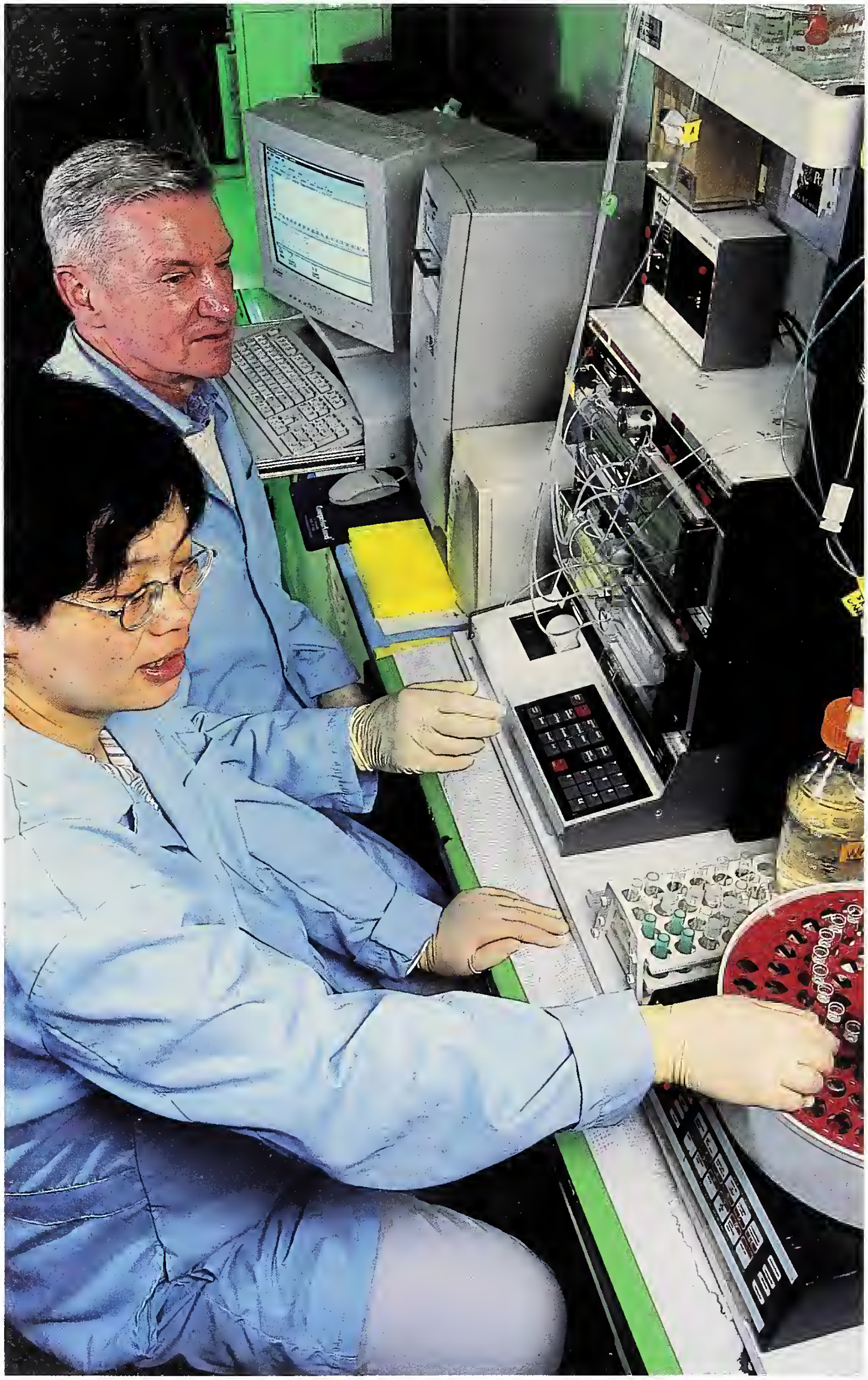
VMRD, Inc., of Pullman, Washington, sells the reagents for the MCF CI-ELISA.

More information on MCF is available at http://www.uwyo.edu/vetsci/mcf_q&a.htm.—By **Marcia Wood,** ARS.

This research is part of Animal Health, an ARS National Program (#103) described on the World Wide Web at http://www.nps.ars.usda.gov.

Hong Li is in the USDA-ARS Animal Disease Research Unit, 3003 Animal Disease Biological Facility, Washington State University, Pullman, WA 99164; phone (509) 335-6002, fax (509) 335-8328, e-mail hli@vetmed.wsu.edu. ◆

PEGGY GREB (K9885-1)



Dairy scientist Max Paape and cell biologist Yan Wang use fast protein liquid chromatography to purify bovine recombinant CD14.

An Udder Solution for Bossie's Woes

It's almost impossible to protect dairy cows from *E. coli* and other coliform bacteria. These gram-negative bacteria lurk in bedding and other damp areas—even in the cleanest dairy barns—waiting for a nice, warm udder to infiltrate. And they make life miserable for 3 million U.S. dairy cows that show visible signs of acute infection . . . not to mention costing an estimated $1.4 billion in annual losses for Bossie's owners from incapacitated cows and milk that can't be sold.

Coliform bacteria account for about 40 to 50 percent of mastitis cases in the United States, and 80 percent of these cows will become visibly sick, says Agricultural Research Service dairy scientist Max Paape. Of the 3 million cows infected annually, the bacteria put about 300,000—or one-tenth—out of commission entirely. And many die from shock induced by the bacterial toxin, or endotoxin.

"Standard therapies haven't been successful in relieving symptoms and reducing mortality from acute coliform mastitis," Paape says. "Vaccines have had limited success in reducing clinical symptoms, but they don't eliminate the coliform organisms."

That's about to change. Late last year, ARS filed a patent application on a recombinant gene that promises both effective treatment for infected cows and prevention in future cows bioengineered with the gene. It codes for a protein—

soluble CD14—that's naturally suspended in cows' milk and blood plasma.

The protein binds to the endotoxin and neutralizes it. That prevents the cow's immune system from overreacting to the toxin, a reaction that bungles the infection-fighting process and could put the animal into shock, explains Paape. He is at ARS' Immunology and Disease Resistance Laboratory in Beltsville, Maryland.

CD14 also sensitizes the lining of a cow's mammary glands to very low levels of endotoxin—produced by just a few bacteria. Once sensitized, these mammary cells start an attack against the infiltrating bacteria before they can get a hoofhold and pour out enough endotoxin to make the cow sick, explains cell biologist Yan Wang, who conducted this research with Paape for her doctoral dissertation. She is now at the National Institute of Allergy and Infectious Diseases in Rockville, Maryland.

Paape and Wang are co-inventors of the recombinant gene and its applications, along with colleague Dante Zarlenga, a molecular biologist who specializes in cloning and designing genes.

A Bet That Paid Off

Paape had already found the CD14 protein embedded in the membranes of white blood cells in cows. And the soluble kind was known to increase during coliform infections in humans and laboratory animals, says Wang. So she and Paape predicted that they'd find the soluble protein in cows' milk. They also bet it could temper the animals' acute reaction to coliform endotoxin while initiating an appropriate response to the infiltrating bacteria. They bet right.

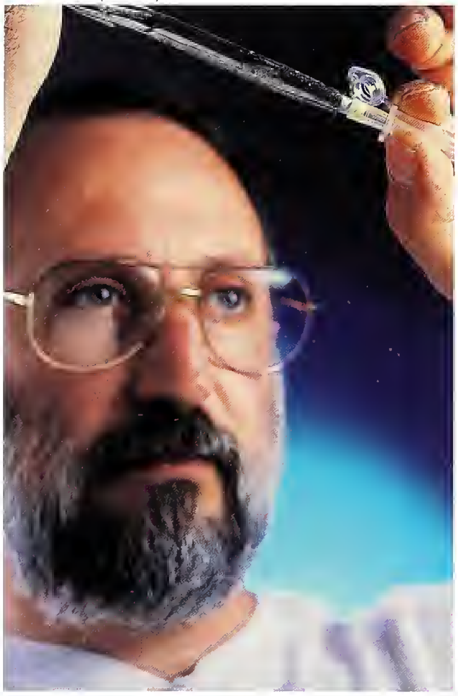
They enlisted Zarlenga's expertise to clone the gene for soluble CD14.

He used as a template the gene for the type of CD14 that stays bound to membranes. Because the gene for the membrane-bound form is longer than the one for the soluble version, he and Wang clipped off the extra bases on one end before inserting the recombinant CD14 gene into bacterial cells.

Then they transferred the gene to insect cells in order to produce enough of the protein to test.

Tissue culture studies showed that the recombinant CD14 protein binds to endotoxin, effectively neutralizing it. The researchers expect it will do the same when injected into a sick cow's blood, but they don't have enough of the protein yet for such a study.

PEGGY GREB (K9886-1)



Molecular biologist Dante Zarlenga performs a step in the process of cloning the gene that codes for CD14, a protein that can neutralize toxins created by mastitis-causing bacteria.

"For the first time, veterinarians will have a product to prevent acute endotoxin shock in dairy cows," says Paape. Wang emphasizes that CD14 "is a protein found naturally in cows, so any side effects should be minimal."

Prevention Preferred Over Cure

CD14 works to prevent infection, too. Paape and Wang incubated the protein with endotoxin in a culture dish to form a complex. When they injected the protein-endotoxin complex into cows' teats, it stimulated the mammary cells to launch an appropriate response—one that brings in the white blood cells that gobble up coliform bacteria—without calling in the cavalry.

"It needs more testing in cows," says Wang, "but I think it's very promising for both treatment and prevention."

While it's not feasible for dairy producers to inject CD14 into their cows' teats regularly, the gene for CD14 can be designed and inserted into tomorrow's dairy cows so that it produces the protein only in their mammary cells. And that's not science fiction.

ARS colleagues in the Gene Evaluation and Mapping Laboratory at Beltsville have already produced a cow with engineered immunity. "Annie" is a clone of a Jersey cow whose mammary cells produce a protein that promises to prevent infections from *Staphylococcus*.

Two of those colleagues, ARS physiologist Robert Wall and support scientist Juli Foster-Fry, are constructing a designer CD14 gene and will insert it into mice. If tests show that it works, Wall plans to insert it into cows. Ultimately, his laboratory wants to bioengineer a cow that is protected against all mastitis-causing organisms. And that's when you'll see Bossie smile!—By **Judy McBride,** formerly with ARS.

This research is part of Animal Health, an ARS National Program (#103) described on the World Wide Web at http://www.nps.ars.usda.gov.

Max J. Paape and Dante Zarlenga are with the USDA-ARS Immunology and Disease Resistance Laboratory, 10300 Baltimore Ave., Bldg. 1040, Room 105, Beltsville, MD 20705-2350; phone (301) 504-8302, fax (301) 504-5306, e-mail mpaape@anri.barc.usda.gov, dzarlenga@anri.barc.usda.gov. ◆

LOST LAMBS

Lessons Learned From Scrapie Outbreak

Katherine O'Rourke isn't the kind of doctor that normally makes house calls. But 2001 was different. That's when O'Rourke, an ARS microbiologist with a Ph.D in veterinary science and immunology, heard about an interesting case in Tucson, Arizona.

It involved a nervous system disease called scrapie that had befallen a flock of about 36 Suffolk sheep managed by José A. Bernal, a science teacher at Amphitheater High School in downtown Tucson. Bernal housed the flock at the school's nearby "lamb lab," a facility where students could gain hands-on experience raising the animals for market and learn about science and agriculture.

But the course became a tough lesson in loss, starting in 1997 when scrapie claimed its first victim: "Baby Face," a prized, 7-year-old pet ewe that Bernal had raised from a lamb. Later, more flock members tested positive for the disease—also known as ovine transmissible spongiform encephalopathy—and, by law, had to be destroyed.

"We ended up getting rid of every single ewe we had that was at risk," says Bernal. "It was bad." So much so, the lamb lab nearly faced closure in 2001. His problems weren't unique, though. By September of that year, 98 other U.S. cases of scrapie had been reported in sheep and 7 in goats. Yearly scrapie losses cost American sheep and goat producers an estimated $20 to $25 million.

Through Bret A. Combs, with APHIS Veterinary Services (VS), Bernal contacted O'Rourke, who was leading a study to decipher the genetic underpinnings of scrapie resistance in sheep at ARS' Animal Disease Research Unit, and Washington State University, both located in Pullman, Washington. There, O'Rourke had also helped pioneer development of a so-called third-eyelid test to detect scrapie's main causative agent: a malformed protein called a prion.

By combining this new, live-animal testing method with sanitation, genetics, and other measures, O'Rourke felt it would be possible to eliminate scrapie from the students' flock. But first she needed to make a house call to better assess the situation. So, in March 2001, she booked a flight to Tucson to meet with Bernal and his students. John V. Duncan, an APHIS-VS collaborator from Casper, Wyoming, went too.

Science in the Classroom

After arriving, says O'Rourke, "We went to the lamb lab and did our live-animal and genotyping tests on the sheep." Armed with the results, she and Duncan later worked up a genetics-based strategy by which Bernal's class could repopulate their flock and eventually certify it as scrapie free.

Future Farmers of America students from Amphitheater High School, Tucson, Arizona, lead sheep from their pens to a site where the animals will be tested for scrapie.

STEPHEN AUSMUS (K10088-1)

For Bernal, it was a golden teaching opportunity, despite the stress of losing several sheep and the lamb lab's near closure. "Our kids had the opportunity to learn firsthand about scrapie and how you go about identifying diseases," says Bernal, who has worked on a cattle ranch and studied animal science in college. "I always want my kids to work with people like O'Rourke who are on the cutting-edge of science," he adds.

One example of this was O'Rourke's use of the third-eyelid test. It's a relatively noninvasive procedure that can detect scrapie-causing prions in young sheep before clinical signs of

STEPHEN AUSMUS (K10086-1)

APHIS veterinarian John Duncan (from Wyoming) clips a tiny piece of a third eyelid from a sheep while students and animal behavior teacher Jose Bernal (wearing striped shirt) hold the sheep.

the disease appear. Around 3 years of age, an infected animal may experience trembling, lip smacking, erratic behavior, and weight loss. Eventually, it becomes so sick it must be destroyed. Through working with Wyoming's commercial wool producers, Duncan and a coalition of federal, state, and university veterinarians were able to extensively field-test the procedure and show its usefulness in screening flocks for scrapie before these clinical signs appear. Their study was published in the journal *Clinical and Diagnostic Laboratory Immunology*.

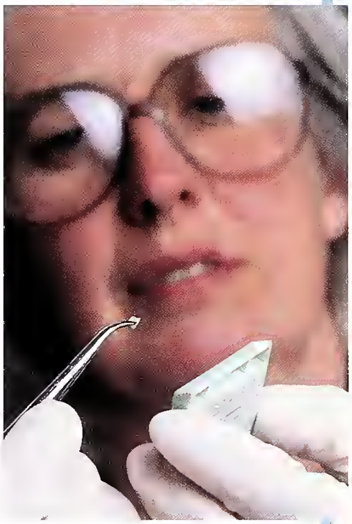
STEPHEN AUSMUS (K10085-1)

William Rivera and Betty Masulis keep a sheep calm while veterinarian John Duncan draws blood to test for genetic resistance to scrapie.

Fine-tuning Flocks With Genetics

This May, Katherine O'Rourke began using genetic testing to an even greater degree in an ARS-funded project led by Robert H. Stobart, along with Gary Moss and Bill Russell at the University of Wyoming's Department of Animal Sciences in Laramie. There, they are evaluating two flocks of sheep representing four breeds—Suffolk, Columbia, Hampshire, and Rambouillet—for traits of economic importance to producers. These include fiber diameter and staple length for the wool breeds, and meat production, weight gain, number of lambs born and weaned, and weaning weights for all the breeds. The researchers will also evaluate lamb performance from weaning to slaughter.

JACK DYKINGA (K8829-1)



Microbiologist Katherine O'Rourke prepares a sample of sheep eyelid tissue for a scrapie test.

The idea is to help producers select sheep with both low susceptibility to scrapie and traits that will turn a profit. This, too, will become increasingly important in the next 10 years as Wyoming and other states and USDA seek to eradicate scrapie, which has hurt U.S. exports of breeder stock, frozen semen, bone meal, and other sheep-derived products.

Meantime, the group plans to compare their project's results with those of Irish researchers who are running a similar study under different conditions. That way, O'Rourke explains, "we get the expertise of another laboratory studying this important question, and we get additional statistical evaluation of the results."

And if time permits, she hopes to make another house call to the lamb lab.—By **Jan Suszkiw,** ARS.

Previously, veterinarians confirmed scrapie by examining tonsil or brain tissue from sheep that had died or been destroyed to prevent them from infecting healthy animals. But with the eyelid test, all that's needed is a small sample of lymph tissue snipped from a special membrane covering the sheep's eye, called the third eyelid. Prions collect on this third eyelid, explains O'Rourke. She helped design a monoclonal antibody that binds to the malformed protein so that it can be identified.

A Primer in Genetics

Genetic testing works differently. Instead of using an antibody to find a protein antigen, this method uses polymerase chain reaction (PCR) and other molecular technologies to home in on a specific gene of interest. In the case of the students' flock, O'Rourke tested for three variations of the gene that codes for the prion protein: 171QQ, 171HH, or 171HQ. Each variant predisposes a sheep to scrapie.

"We break apart, or lyse, white blood cell samples with detergent and then remove their DNA," O'Rourke explains. "We use PCR to locate the prion gene and then use an enzyme and primers to replicate the DNA region in which the gene resides.

Once the gene segment is reproduced, it is labeled with chemical "letters" called nucleotides. The result is a complementary sequence of letters that serves as the prion protein's calling card—and the sheep's susceptibility to scrapie.

In Baby Face, the gene variants 171QQ or 171HQ probably predisposed her to the disease by enabling the prion protein to replicate in her brain and lymph tissues. "A sheep might be genetically predisposed to scrapie, but the scrapie agent has to come in from the outside," notes O'Rourke. There are different ways Baby Face could have become infected. One possibility is that she grazed an area where an infected ewe had given birth, aborted, or deposited placental material containing the prion.

The prion has a shape-shifting nature that makes it toxic to the animal. "It takes on the look of a pleated sheet rather than a smooth helix," she notes. Some sheep, however, are endowed with beneficial forms of the gene—dubbed 171QR or 171RR—that actually prevent such shape-shifting. "We know that the 171R variant works, but we don't know how," says O'Rourke. Researchers are still debating whether or not to use the term "resistant" to describe such animals.

Either way, "it's a lucky break for sheep that have this gene," O'Rourke adds. But only about half of any given flock is likely to harbor the 171QR/RR variant, she adds. With genetic testing, though, producers may soon be able to tip the scales in their flock's favor by checking for the protective gene in rams used for breeding.

So far, the strategy seems to be working for Bernal's class. Their flock now boasts 35 ewes and 2 rams and, as of March, the sheep have been deemed scrapie free by inspectors.

Once again, Bernal's class plans on showing their sheep at the state fair, and he has high hopes they'll become Arizona's "first scrapie-free flock with a protocol in place" for preventing the disease.

"The whole thing is a great story," says O'Rourke of the students' efforts. "It's also a small-scale example of what's going on around the country to control scrapie."—By **Jan Suszkiw,** ARS.

This research is part of Animal Production, Product Value, and Safety, an ARS National Program (#103) described on the World Wide Web at http://www.nps.ars.usda.gov.

Katherine O'Rourke is in the USDA-ARS Animal Disease Research Unit, P.O. Box 646630, Washington State University, Pullman, WA 99164; phone (509) 335-6020, fax (509) 335-8328, e-mail korourke@vetmed.wsu.edu. ◆

STEPHEN AUSMUS (K10087-2)

STEPHEN AUSMUS (K10087-1)

Top: Students watch as APHIS veterinarian John Duncan performs a test for scrapie susceptibility. Future Farmers of America members are wearing blue shirts. Bottom: The group eagerly awaits the test results—samples turning blue indicate susceptibility.

Sequencing the Bad Guys

A high-tech piece of equipment, hard work, and collaboration have brought researchers a few steps closer to developing new tests and vaccines for several troublesome cattle diseases.

Scientists at ARS' Bacterial Diseases of Livestock Research Unit in Ames, Iowa, and the University of Minnesota (U-M) have sequenced the chromosomes of two disease-causing microbes—those that cause Johne's disease and bovine brucellosis—and have moved into the finishing phase of work on an agent that brings about leptospirosis. That project is being done completely within the ARS unit, which is part of the National Animal Disease Center (NADC).

The arrival at Ames of a DNA sequence analyzer has made much of this work possible. "Our sequencing capacity was a limiting factor for large-scale projects," says ARS veterinary medical officer David Alt, who operates the analyzer. "Now we can perform almost 800 reactions a day." The sequencer can automatically analyze multiple runs of 96 DNA samples, making unattended 24-hour operation possible.

Alt compares a sequenced genome to a book. "We can read the book from start to finish," he says. "We are not going to understand every word in it, but it's a starting point that may lead to treatments, vaccines, and diagnostic means that are better than those currently available."

Automated sequencing allows for rapid analysis of an organism's genes, speeding identification of those linked to superior characteristics or to negative traits such as susceptibility to disease. Scientists and breeders can then, theoretically, root out or exploit specific genes to breed improved varieties or species. The Ames/U-M research seeks to identify genes that are associated with disease and that show potential in vaccine development.

NADC's first sequencing project was on *Mycobacterium paratuberculosis*. That microbe causes Johne's disease, an intestinal disorder characterized by diarrhea and weight loss in infected cattle and found in 7 percent of beef herds and 22 percent of dairy herds nationwide. ARS microbiologist John Bannantine and Vivek Kapur, a U-M pathogenomics scientist, led this research.

Microbial causes of some livestock diseases are among the latest organisms to be genetically **mapped.**

"With the sequencing," says Bannantine, "we hope to understand the biology of *M. paratuberculosis,* identify unique disease-causing genes, and—most importantly—develop new diagnostic tests."

The maps are a starting point that may lead to treatments, vaccines, and diagnostic means for **Johne's disease** and **bovine brucellosis.**

ARS microbiologist Shirley Halling led sequencing work on *Brucella abortus,* which causes bovine brucellosis. This highly contagious bacterial disease induces late-term abortions and infertility in cows. It can also bring about undulant fever in humans.

The sequencing of *Leptospira borgpetersenii* serovar *hardjo*, a cause of leptospirosis, is being led by ARS microbiologist Richard Zuerner. Leptospirosis causes abortions, stillbirths, and weak offspring in cattle and swine and can reduce milk production in cows. It also affects many other animals, including dogs, and is an important public health concern.—By **Luis Pons,** ARS.

This research is part of Animal Health, an ARS National Program (#103) described on the World Wide Web at www.nps.ars.usda.gov.

David P. Alt is with the USDA-ARS National Animal Disease Center, 2300 Dayton Ave., Ames, IA 50010; phone (515) 663- 7645, fax (515) 663-7458, e-mail dalt@nadc.ars.usda.gov. ★

An Easy, Inexpensive Test Detects Tuberculosis in Livestock and Wildlife

The United States has come close to eliminating bovine tuberculosis (TB). Before USDA launched a tuberculosis eradication program in 1917, 5 percent of the nation's cattle were infected. That figure dropped to 0.015 percent by 1990.

But recent developments demonstrate that *Mycobacterium bovis*, the causative agent of bovine TB, is far from eliminated. In Michigan, an outbreak among white-tailed deer that may have started in livestock has spread to at least 20 cattle herds. Importation of Mexican feeder cattle has sparked concerns in Texas, where two herds have tested positive. In both states, the disease has led to economic woes and forced depopulation of infected herds.

That is why an invention by scientists at ARS' National Animal Disease Center in Ames, Iowa, may be not only revolutionary, but also very timely.

PEGGY GREB (K10096-1)

Veterinarian Ray Waters collects a blood sample from an elk for the nitric oxide assay, a new test for detecting tuberculosis in animals.

The breakthrough—a new blood-based test for detecting TB in animals—is important because it is applicable for most if not all species of mammals, and requires only a single blood sample. That means animals are handled just once rather than twice.

Veterinarians Ray Waters and Mitch Palmer, who work in the center's Bacterial Diseases of Livestock Research Unit, have submitted a patent application for the assay. Developed over 2 years, the invention is an inexpensive and easy process to be used mainly by diagnostic laboratories and regulatory agencies, says Waters.

Currently, the only government-approved TB-detection method is the cumbersome skin test.

"With it," says Waters, "a crude mixture of tuberculosis antigens is injected into the skin of the animal, and any ensuing reaction must be measured 72 hours later. When the animal is handled again, it can lead to injury and stress, especially to wildlife species."

The TB bacterium can be inhaled or ingested and is spread mainly through the respiratory and lymphatic systems. It exists in three main types: human, avian, and bovine. Bovine TB can infect most mammals, including wildlife.

Palmer says the new, still-unnamed test can detect all three types as long as proper antigens are used. The assay may even be used to discriminate between bovine and avian TB, although further studies are needed.

He says that another test, an interferon gamma assay already in use for livestock, is based on the same blood-culture principle as their procedure. But it cannot be applied to other species and can be used only in conjunction with the skin test.

According to Waters, the invention will likely be used to detect TB in livestock species such as cattle, sheep, and goats, as well as in wildlife species such as deer, bison, and elk. It can also work for humans, he says, although it is not an adequate replacement for the current tests.

Nitric Oxide Is the Key

The test detects nitrite, as an indication of nitric oxide production, in blood-sample cultures. Mammals produce nitric oxide as a natural response when fighting TB. While the interferon gamma assay uses species-specific monoclonal antibodies, the new test uses a detection method that will likely work for many mammals. This is possible because nitrite is a chemical easily detected within samples from all species.

The interferon gamma assay currently in use measures a chemical messenger produced by white blood cells fighting TB and other infections. Interferon gamma, unlike nitrite, differs between species, so new reagents are needed for each species tested.

PEGGY GREB (K10098-1)

Technician Theresa Rahner begins to process an elk blood sample for the nitric oxide assay.

PEGGY GREB (K10094-1)

A couple of white-tailed deer from the National Animal Disease Center research herd feed from the hand of Ray Waters.

Concern over the spread of bovine TB goes beyond cattle and profits. "It is a public health concern," says Palmer. "As an example, before the eradication program and before milk was pasteurized, 20 to 30 percent of tuberculosis cases in humans came either from contact with cattle or from drinking infected milk. We've almost eradicated that threat here, but bovine TB is still a public health issue in other countries."

A Timely Test

The invention comes at a time when livestock owners in Michigan and Texas are contemplating the effects of bovine TB on business.

"When a farmer or a rancher discovers TB in his herd, animal movement stops," Palmer says. "Other states are not going to allow those infected cattle in. It also affects animal trade internationally."

He notes that infected herds face destruction or quarantine for an extended and costly period that is followed by retesting. Waters says the outbreak has cost Michigan more than $50 million in increased testing and lost trade.

Palmer says the disease can spread from mammal to mammal through contact with saliva, nasal excretions, urine, and feces. "In the case of cattle, it occurs when deer enter the areas where cattle are raised and fed," he says.

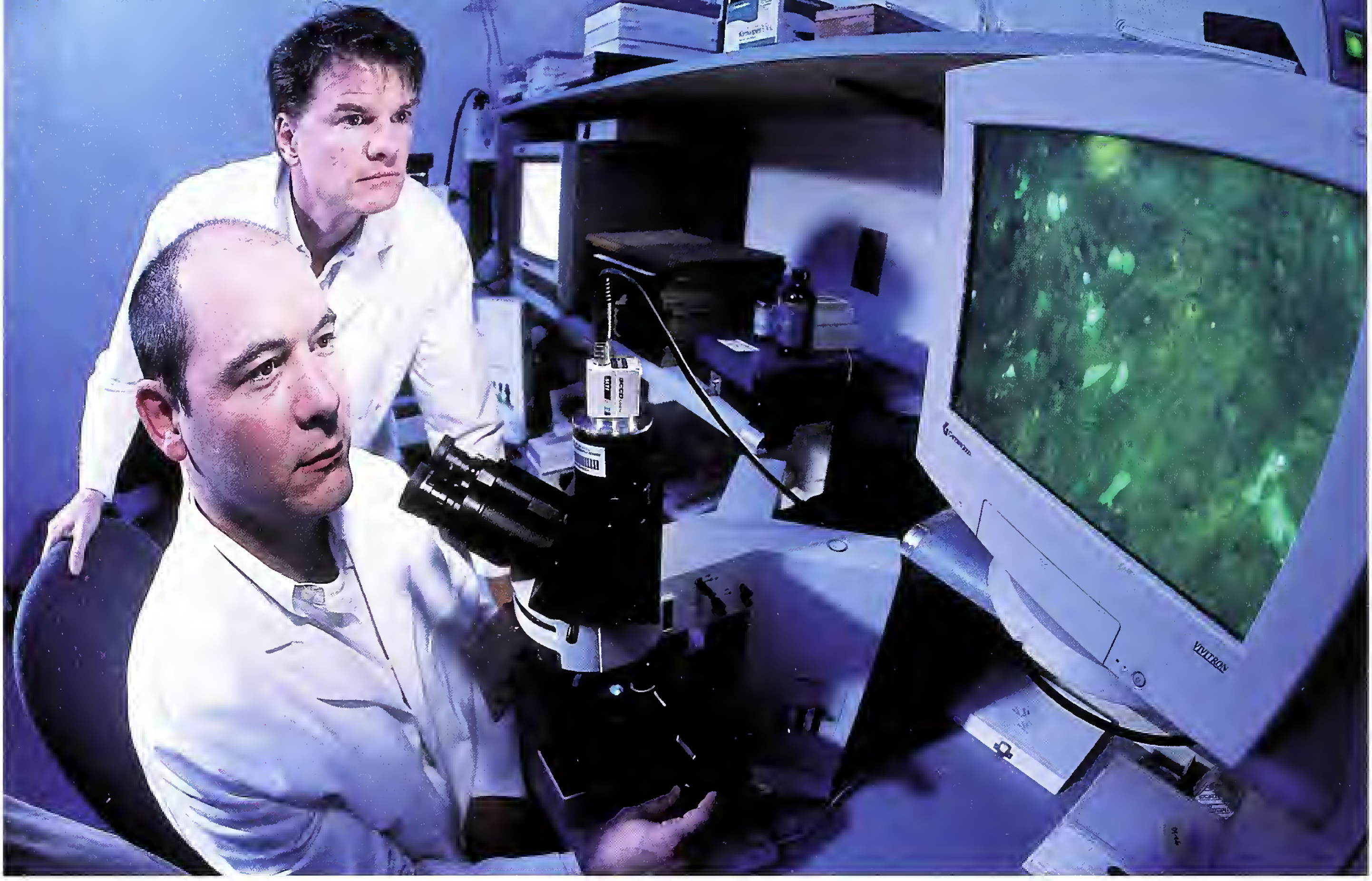
Waters says the new assay, which was tested mainly on white-tailed deer, will be applied mostly to captive wildlife and livestock. "The method will not be used for testing of wild deer," he says. "It will help with monitoring animals that are moved—particularly across borders—to make sure the disease doesn't go undetected."

The invention may prove useful on species usually found in the wild but kept captive and transported for reasons related to food, hunting, and research. It may also help zoos, where, Palmer says, "tuberculosis is a bigger problem than you might think. That is especially true with animals coming from countries where TB is endemic. This test can be run on samples from animals before they are brought into the country or shared with other zoos."

The test should be a decisive weapon in the fight against a disease once thought defeated that has instead shown alarming persistence.—By **Luis Pons,** ARS.

This research is part of Animal Health, an ARS National Program (#103) described on the World Wide Web at http://www.nps.ars.usda.gov.

Wade R. Waters and Mitchell V. Palmer are with the USDA-ARS National Animal Disease Center, Bacterial Diseases of Livestock Research Unit, 2300 Dayton Ave., Ames, IA 50010; phone (515) 663-7756 [Waters], (515) 663-7474 [Palmer], fax (515) 663-7458, e-mail rwaters@nadc.ars.usda.gov, mpalmer@nadc.ars.usda.gov. ◆



STEPHEN AUSMUS (K10699-1)

Microbiologist Darrell Kapczynski (foreground) and immunologist Terry Tumpey use ultraviolet microscopy to examine cell monolayers for production of a green fluorescent protein that indicates binding of the vaccine to host cells.

An END for Exotic Newcaastle Disease Virus?

The last thing poultry producers want their flocks hit with is exotic Newcastle disease (END), a contagious and fatal viral disease affecting most species of birds. Almost all unvaccinated chickens die within days of being infected with the virus.

The END outbreak, diagnosed in late 2002, left its mark on California, then spread to Nevada and Arizona before it was contained. About 3.5 million commercial and backyard poultry, including chickens, geese, peacocks, pigeons, and turkeys, were euthanized to stop the disease from marching into other states. More than $104.5 million has been spent by a state-federal task force to try to contain and eradicate END.

To prevent a devastating outbreak in commercial flocks in the United States, scientists are researching diagnostic tools as well as preventive vaccines. Agricultural Research Service microbiologist Darrell Kapczynski is working on a new type of vaccine to combat the virus.

The vaccines currently available for Newcastle disease virus are made with either an attenuated (weakened) live virus or a killed virus. Either type stimulates an immune response in the bird, which protects it from future exposure to the virus. While these vaccines are effective, some production losses have been attributed to the live ones, and the inactivated ones are more expensive to administer. To overcome those problems, Kapcyznski and his colleagues at the Southeast Poultry Research Laboratory in Athens, Georgia, developed what's known as a nonreplicating virosome vaccine.

"Essentially, the virus is taken apart, the replicating genetic material is removed, and the virus is put back together," explains Kapczynski. "This vaccine induces protective immunity but does not allow the virus to replicate—copy itself—or pass from bird to bird."

The virosome vaccine is composed of liposomes, water-insoluble spheres encased in lipid layers. The liposomes contain certain viral protein antigens but not the virus replication machinery. The antigens are able to bring about a protective immune response in the animal.

In one study, day-old chicks were divided into three groups: a control group, which received saline solution; a group that received live-virus vaccine; and a group that received the virosome vaccine. Two weeks later, birds were challenged with a lethal dose of the virus. All birds were monitored daily for clinical signs of disease and mortality. Birds in the control group did not survive the challenge, but birds that received either the live-virus or virosome vaccine were 100 percent protected from the END virus.

While the cost of virosome technology is currently prohibitive, there are several potential advantages. First, since the vaccine has no replicating genetic material, the virus can't mutate or transfer from bird to bird. Second, since

STEPHEN AUSMUS (K10702-1)

Technician Tracy Smith-Faulkner examines chicken embryos for the presence of virus in samples collected from vaccinated birds. The absence of virus in embryos indicates the birds were protected against infection and disease.

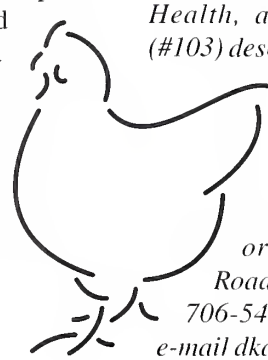
STEPHEN AUSMUS (K10700-1)

A virosome vaccine against Newcastle disease is administered to a baby chick intranasally by microbiologist Darrell Kapczynski.

the virosomes are able to attach and fuse with host cells, as would the live virus, a strong immunity is induced. Third, it is possible to differentiate between vaccinated and virus-infected birds. Birds vaccinated with an attenuated live or a killed virus will produce antibodies against all the virus's proteins. This leaves producers unsure of whether the flock is infected by field (nonvaccine) virus. But virosome vaccines induce antibodies against only two END proteins—the fusion and hemagglutinin-neuraminidase proteins. This allows producers to identify vaccinated flocks by testing for antibodies against these proteins. Birds exposed to field virus can be identified by testing them for antibodies to viral proteins not included in the virosome. Also, production losses attributed to using a live-virus vaccine are not an issue when using virosomes.

Under certain circumstances, vaccinating a flock does not guarantee complete protection. Kapczynski and colleague Daniel (Jack) King studied commercial birds that were vaccinated with a commercial vaccine against Newcastle disease and then exposed to the END virus. Seventy-five percent of the flock died. "It took longer for the birds to get sick and die," says Kapczynski. "It seems that even though the birds had been vaccinated, they were severely weakened by the virus challenge." In the field, weakened birds are much more susceptible to infection by secondary pathogens.

Even though END has not spread widely throughout the United States, it is still necessary to find ways to protect commercial and backyard poultry flocks and indigenous birds. The recent isolation of END virus from a backyard flock in Texas underscores the need for continued surveillance.



The next step for Kapczynski and his colleagues is to determine whether the virosome vaccine can protect a typical commercial flock, which is exposed to various production and environmental stresses, such as other illnesses and temperature fluctuations. "It's a long way from the lab to the field. The vaccine has to be protective in the field, which is the gold standard of effectiveness," says Kapczynski.

Successful completion of this work may offer poultry producers a new option for ending END.—By **Sharon Durham,** ARS.

This research is part of Animal Health, an ARS National Program (#103) described on the World Wide Web at www.nps.ars.usda.gov.

Darrell Kapczynski is with the USDA-ARS Southeast Poultry Research Laboratory, 934 College Station Road, Athens, GA 30605; phone 706-546-3471, fax 706-546-3161, e-mail dkapczynski@seprl.usda.gov. ★

Reverse Process May Be Key To Developing Swine Flu Vaccine

Technician Deborah Clouser prepares purified plasmids containing individual swine influenza A virus gene segments for constructing a live swine influenza A virus.

In studies to construct a swine flu vaccine, cells are observed for signs of change that indicate a live swine influenza A virus was generated by reverse genetics.

SCOTT BAUER (K11003-1)

ARS scientists studying a strain of swine influenza new to this part of the world have found that taking one step backward can lead to many steps forward.

Veterinary medical officers Jürgen Richt and Kelly Lager of the ARS National Animal Disease Center (NADC) in Ames, Iowa, are using a process called reverse genetics to gain insight into an alarming development: rapid spread throughout North America of a swine flu type that contains gene segments from birds and humans as well as from pigs.

The researchers, who work in NADC's Virus and Prion Diseases of Livestock Research Unit, are using reverse genetics to create new flu viruses in efforts to explore individual components of the virus. The hope is that these components can in turn be exploited by vaccines.

Reverse genetics has been developed over the past decade for virus studies. "The technology has now advanced to where one can confidently generate influenza viruses entirely from cloned DNA resulting from the process," Lager says. Richt says this work is unique because, while the process has been used on human flu strains, it has never been used on swine flu.

Swine influenza presents a special challenge in genetics because its genome comprises eight segments of ribonucleic acid, better known as RNA. RNA viruses such as swine flu store their genetic information in RNA, which is more susceptible to mutation than DNA. This allows RNA viruses to evolve far more rapidly than DNA viruses and sometimes makes it hard for an infected host to develop lasting immunity.

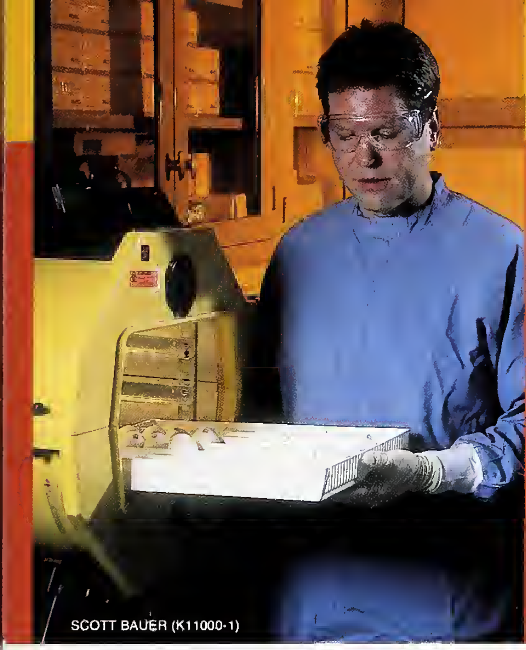
What's "Reverse" Got To Do With It?

What made reverse genetics attractive for exploring the new swine flu strains is that manipulations commonly done on DNA cannot be performed with RNA.

That's where the "reverse" in reverse genetics comes in. "Scientists can convert RNA viruses' genetic material into a DNA state," says Richt. "This is called reverse transcription. At this point, mutations can be introduced into the resulting cloned DNA. Once the DNA is converted back into RNA, the introduced mutations will occur in the genome of the RNA virus. Through this approach, we use cloned DNA to generate swine influenza viruses," says Richt.

Richt and Lager—collaborating with veterinary pathologist Bruce H. Janke of Iowa State University and virologist Richard J. Webby of St. Jude Children's

Swine influenza is an acute respiratory disease of swine whose symptoms include anorexia, fever, depression, coughing, and troubled breathing. It is among the type A influenza viruses, which can affect humans as well as chickens, ducks, horses, seals, whales, and other animals.



Veterinary medical officer Jürgen Richt removes embryonated chicken eggs from an incubator. These eggs are used to propagate swine influenza A viruses.

Jürgen Richt (left) and veterinary medical officer Kelly Lager use a laryngoscope to inoculate an anesthetized pig, while Deborah Clouser observes.

Research Hospital in Memphis, Tennessee—generated the A/Swine/Texas/4199-2/98 virus, or TX/98 for short.

"When tested in experimentally infected pigs, this generated virus showed characteristics similar to its parental wild type," says Richt. In addition, pigs infected with TX/98 viruses that were genetically altered through mutation or deletion showed significantly less evidence of flu infection, says Lager. "This makes these viruses potential candidates for modified-live vaccines."

Swine influenza is an acute respiratory disease of swine whose symptoms include anorexia, fever, depression, coughing, and troubled breathing. It is among the type A influenza viruses, which can affect humans as well as chickens, ducks, horses, seals, whales, and other animals.

New Flu Strain Changes Everything

Specialists in North America used to diagnose almost exclusively only one type of flu virus in pigs: H1N1. That changed in 1998, when pigs started to be diagnosed with H3N2, a strain that up to that time was rarely seen here.

Since then, Lager says, these H3N2 viruses have combined, or reassorted, further with the classical H1N1 viruses, resulting in new H1N2 and H1N1 swine influenza viruses. The increased virulence represented by this new strain also raised concerns, he adds.

The H3N2 virus appeared in two types: a double reassortant (DR), labeled as such because it contains gene segments from both human and swine flu; and a triple reassortant (TR) that also contains gene segments from avian viruses. Richt says it's the TR viruses that are causing most of the trouble. "By the end of 1999, these had spread throughout the United States, whereas the DR viruses had not," he says.

Birds play an important role in the flu dynamic, providing a global reservoir of A-type viruses. It is believed flu resides harmlessly in birds, where viruses are genetically stable. When a virus from birds infects pigs that are already infected with a swine influenza virus, gene segments from each virus can be mixed, and a new influenza virus can arise. This reassortment likely produced the TR H3N2 virus.

Richt and Lager believe reverse genetics can greatly benefit the study of influenza in humans as well as in pigs.

"Current human and swine vaccines are inactivated vaccines that vary in efficacy, depending on the match of the vaccine with influenza virus strains circulating in the susceptible population," says Richt. "Modified-live-virus vaccines generated through reverse genetics can stimulate a better, broader immune response than killed-virus vaccines."

There is a trade-off, however, in that modified-live-virus vaccines may not be as safe as killed-virus vaccines. "There's always a chance a modified-live-virus vaccine may gain virulence as it replicates in the vaccinated host," Richt says.

"But if a modified-live-influenza-virus vaccine can be developed, it may be an important tool in preventing flu in pigs and humans. We also believe reverse genetics can contribute to our understanding of how influenza virus causes disease in various host species."—By **Luis Pons,** ARS.

This research is part of Animal Health, an ARS National Program (#103) described on the World Wide Web at www.nps.ars.usda.gov.

Jürgen A. Richt and Kelly M. Lager are in the USDA-ARS Virus and Prion Diseases of Livestock Research Unit, National Animal Disease Center, 2300 Dayton Ave., Ames, IA 50010; phone (515) 663-7366 [Richt], (515) 663-7371 [Lager], fax (515) 663-7458, e-mail jricht@nadc.ars.usda.gov, klager@nadc.ars.usda.gov. ★

KEITH WELLER (K9144-1)

ARS dairy scientist Albert Guidry credits cooperation among ARS, state universities, dairy producers, and industry for the success of the *Staphylococcus aureus* vaccine against mastitis.

Promising New Mastitis Vaccine

Udderly stubborn staph succumbs to test vaccine. But will it prevent infection?

Al Guidry is looking for a herd of willing heifers.

He could use about 1,000 of these adolescent cows to test a new vaccine against the toughest form of mastitis—the kind caused by *Staphylococcus aureus*. Current commercial vaccines immunize against two staph strains that cause only about 40 percent of staph-induced mastitis cases in the United States, slightly more in Europe. And antibiotics are ineffective against staph because the bacteria have become resistant, or they have holed up in regions of the gland where the drugs can't reach.

So Guidry, a dairy scientist at the Beltsville (Maryland) Agricultural Research Center, went looking for the missing links. He screened 44 percent of the U.S. dairy herd to find the serotypes responsible for the other 60 percent of staph-related mastitis cases.

Collaborator Ali I. Fattom, with the biotechnology company Nabi in Rockville, Maryland, had what Guidry was looking for: a single serotype of *S. aureus*—called 336. Fattom, who is involved in developing a human vaccine against staph, knew that 336 accounts for only about 10 to 12 percent of human staph infections. In U.S. cows, however, it's responsible for 50 to 60 percent.

The result of this collaboration is a trivalent vaccine containing 336 together with the other two staph strains known to cause mastitis. Whether or not the new vaccine will prevent mastitis still needs to be proved. But it can cure it—even a good percentage of the most recalcitrant cases—when combined with antibiotics. That's according to tests being led by another of Guidry's longtime colleagues, Michigan State University veterinary scientist Phil M. Sears.

Sears had been looking for a way to boost the bovine immune system, hoping that a more vigorous immune response combined with antibiotics would control chronic mastitis. And he had good results. When he isolated the causative *S. aureus* strain from a dairy herd, killed it, then injected it back into the infected cows a few weeks before administering antibiotics, he cured more than half.

But isolating the causative agent from each herd is too cumbersome for commercial use. The trivalent vaccine appears to solve this problem.

"It applies to all herds, and it's a purer, cleaner preparation," says Guidry. When Sears tested it in commercial dairy cows, it proved to be as effective as his herd-specific vaccine, curing 55 to 60 percent of infected cows.

Because of these promising results, Sears is confident the vaccine will protect heifers from infection. "I don't have any doubt," he says, noting that the vaccine cleared staph infections in about 10 percent of infected cows—even before he administered antibiotics. And it cleared 7 of the 9 cases in the Beltsville herd with the administration of antibiotics, Guidry adds.

Nabi and ARS are jointly applying for a patent covering the new vaccine. The company will look for a partner with channels in the agricultural arena to fund further studies and market it. Before the vaccine can go to market, its ability to prevent infection needs additional validation, says Guidry.

He figures that will require several years and a whole lot of heifers.—By **Judy McBride,** ARS.

This research is part of Animal Health, an ARS National Program (#103) described on the World Wide Web at http://www.nps.ars.usda.gov.

Albert J. Guidry is with the USDA-ARS Immunology and Disease Resistance Laboratory, 10300 Baltimore Ave., Bldg. 173, Room 105, Beltsville, MD 20705-2350; phone (301) 504-8285, fax (301) 504-9498, e-mail aguidry@lpsi.barc.usda.gov. ◆

ARS Research Targets Bovine Viral Diarrhea and Other Pestiviruses

A new type of pestivirus in wildlife has been identified by Agricultural Research Service scientists. "Pestivirus" is a scientific term for a group of viruses that includes economically important ones such as bovine viral diarrhea (BVD) viruses and hog cholera virus, also known as classical swine fever virus. Pestiviruses can also cause reproductive failure and congenital defects in ruminant animals.

Microbiologist Julia F. Ridpath and others at ARS' National Animal Disease Center (NADC) in Ames, Iowa, characterized the new virus, which was isolated from antelope tissues by Wyoming State University researchers.

"While no disease is yet associated with the new pestivirus, its presence in wildlife is significant because wildlife come in close contact with domestic livestock and can transmit disease," says Ridpath. The identification of this new pestivirus is the result of ongoing research being done at NADC to improve the detection and control of pestiviruses.

Research on pestiviruses dates back to the 1930s, when USDA researchers showed that hog cholera was caused by a virus. They developed a test and a vaccine that led to eradication of hog cholera in the United States in 1978.

Current pestivirus research at NADC focuses on BVD viruses, which circulate in cattle herds, leading to lower milk production, poor feed conversion, and significant reproductive problems. They are the most important enteric viral agents of cattle in the United States. Although many commercial vaccines exist for BVD viruses, they continue to be one of the most costly disease problems facing U.S. cattle producers. Losses could be reduced if a quick, reliable, and technically simple test were available to field veterinarians.

With the goal of producing improved vaccines and diagnostics, ARS and ImmuCell Corporation of Portland, Maine, have entered into a research agreement to develop quicker, field-ready tests for detecting BVD viruses. And ARS and Intervet, Inc., of Millsboro, Delaware, have entered into a research agreement to develop a new, more effective vaccine for BVD viruses.—By **Linda McGraw,** ARS.

Julia F. Ridpath is in the USDA-ARS Virus and Prion Diseases of Livestock Research Unit, National Animal Disease Center, P.O. Box 70, Ames, IA 50011; phone (515) 663-7372, fax (515) 663-7458, e-mail jridpath@nadc.ars.usda.gov. ◆

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Animal Agriculture

The major focus of agriculture is the need to increase food production to meet a growing world population. In the United States, for example, two-thirds of our protein consumption and one-third of our energy requirements are met by foods of animal origin. The world is moving towards a global market. Many peoples of the world have profited from free market trade and have become more affluent as a result. These people want more access to animal products. As a result of increasing demand for access to animal protein and as per-capita income rises in developing and industrialized nations, there is an escalating market, both national and international, for foods of animal origin. In some areas of the world, the demand will be hard to meet because of constraints on animal health caused by disease. Advanced molecular and immunologic tools and a new understanding of genomics offer us a greater hope for finding ways to more effectively produce nutritious and safe food for a rapidly expanding global population.

Animal disease is the single greatest hindrance to efficient livestock and poultry production on a global basis. The U.S. livestock industry is a multibillion-dollar industry with yearly farmgate receipts of $96.8 billion, and animal products account for over half of farmgate receipts from all agricultural products. In the past 20 years, the rise in metric tons of food animals produced in the United States has been extraordinary. The value of animal production to the U.S. economy has equally skyrocketed.

Animal Disease Limits Animal Agriculture

The cost of disease in livestock and poultry has been estimated to be as much as 17 percent of production costs in the developed world, and more than twice this figure in the developing world. Though we pride ourselves on the high level of human and animal health care available in our country, losses from livestock disease cost our economy billions of dollars each year. Animal disease threats are more important now than ever before, because of the loss of genetic diversity in herds and flocks and more efficient management practices that concentrate larger numbers of animals into smaller areas. These cost-efficient management practices may place animals at a greater risk for severe disease outbreaks, yet these very practices also help to prevent the introduction and spread of many infectious diseases, because of improved housing, sanitation, biosecurity, and implementation of sound principles in management and building design.

To maintain a cost-effective animal agricultural system that meets high international standards and the expectations of today's consumer, the United States will need to develop new animal disease control strategies and improved management practices. A whole new array of tools to control animal disease is required. Some of these new approaches will come about as a result of the need to remain economically competitive in a global marketplace. Others will come about as a result of loss of the use of antimicrobial agents and limited products available for disease prevention and treatment in animal agriculture. Certain pharmaceutical products used in animal agriculture, such as antibiotics and pesticides, are not likely to be replaced with new and improved products, because of the enormous cost of developing new drugs, the limited market,

and small profit margins. The potential loss of the use of antibiotics and pesticides to protect animal health is alarming. There will be fewer options to prevent important animal diseases such as pneumonia, diarrhea, mastitis, and skin and reproductive infections. Due to prohibitions on drug usage, animal well-being may be seriously compromised. Animals will potentially be infested with larger numbers of ticks and bothered by larger numbers of flies. Not only will these issues affect animal health and well-being but they will also potentiate the emergence of new zoonotic diseases, giving rise to increasing incidence of public health problems.

Challenges of Organic Agriculture in Animal Health

Organic agriculture is on a fast track to keep up with new product demands from consumers. American consumers are becoming more interested in purchasing organic products despite higher cost. Niche markets are emerging and offer new opportunities for farmers. Trying to raise animals using organic standards poses new and sometimes complex challenges for producers. Organic farmers are turning to animal health researchers and are urgently demanding new methods to prevent disease and the loss of production that often results from disease. New means to control internal and external parasites of animals will need to be developed. Alternatives to treating animals with antibiotics and pesticides will require new approaches and new investments in research to find new solutions for disease control.

Market-Driven Integration of Animal Agriculture

Animal agricultural production practices are rapidly evolving toward greater integration and larger farming operations utilizing intensive management practices. Livestock may be transported from one management unit to another following weaning and during the growth and reproductive cycle, thus mixing animals from different points of origin. Livestock raised on the same premises from birth to slaughter are no longer the norm but the exception. Animals can be moved hundreds to thousands of miles in a matter of hours. More cost-effective production has led to vertical integration of the poultry, swine, and dairy industry, and some aspects of the beef cattle industry. Today's food safety standards and consumer expectations for a safe and wholesome food supply are forcing producers to develop and utilize new disease management practices emphasizing HACCP and biosecurity. Food safety and the desire for uniform product quality have required animal industry groups to move rapidly toward integration of production. By controlling the process from birth to market, the animal industry can more easily ensure the safety, uniformity, and quality of the product. Management practices are becoming more widely divergent between large integrated operations and small farm operations, thus posing very different disease control issues. This difference in production practices challenges animal disease researchers. It means that they must keep a broader focus, address disease issues associated with each type of operation, and provide cost-effective solutions for every U.S. animal production unit.

Impact of Emerging and Re-emerging Pathogens on Animal Health

During the past 10 years, emerging and re-emerging pathogens have become a major human and animal health concern. Several new emerging animal disease issues appear every year. Globalization of trade, movement of masses of people and agricultural products, changing weather patterns, rapid population growth in cities, intensive agriculture, limited genetic diversity in farm animals, changes in farm practices—all these factors are creating new opportunities for the re-emergence and spread of infectious diseases, including those resistant to antibiotics in both humans and livestock. Exotic (non-native) organisms, once introduced into the United States, can escalate into an epidemic because of the absence of vaccines or effective drugs, lack of resistance in host animals, and limited resources to effectively manage the spread of such pathogens.

Timely and effective control strategies are needed to avoid economic disruptions and maintain consumer confidence in the ability of the Federal and State governments to handle animal health emergencies. The presence of bovine spongiform encephalopathy (BSE) in the United Kingdom and the recent resurgence of classical swine fever (hog cholera) in Europe have had a profound impact on the agricultural economy of a number of countries and have resulted in trade embargos. The outbreak of foot-and-mouth disease in Taiwan, Korea, and Japan has had a similar impact on the agricultural economy of those countries and has closed Taiwan's export markets. In all of these recent disease outbreaks, millions of head of livestock have been destroyed, creating new environmental concerns about the safe disposal of diseased carcasses. The United States can ill afford an outbreak of a catastrophic disease that could disrupt our national food supply and export markets. For diseases to be effectively detected and controlled, the biology and ecology of the causal pathogens must be understood and weaknesses exploited to limit their spread. Rapid diagnostic tests, novel genetic vaccines, immune modulation strategies, disease resistance genes, and increased biosecurity measures will be needed to prevent or control outbreaks and the spread of animal diseases.

Since nearly 200 zoonotic diseases can be naturally transmitted from animals to humans, expanded research is needed to accelerate the development of knowledge and technologies to protect the health of livestock, poultry, wildlife, and humans in the United States against zoonotic diseases. In recent years, three out of four newly recognized emerging diseases of humans have been traced to animal origin.

Impact of Animal Health on International Trade

Increased opportunities for agricultural trade are demanding new animal disease control standards. Trading practices are easily affected by animal diseases and have been used as trade barriers to protect a country's livestock and poultry. Despite agricultural trade barriers, the quality of U.S. livestock products is globally recognized, and the export of U.S. animal products is on the rise worldwide. In 1999, the United States

exported approximately $57 billion in agricultural, fish, and forestry products, of which approximately $9.8 billion was of animal origin. Exports of total poultry meat and pork have been steadily increasing. In 2003, U.S. exports of agricultural, fish, and forestry products were valued at $68 billion. The poultry industry exports about 14 percent of total poultry meat products. The potential loss of access to international markets is a rising concern and places even greater emphasis on the need for greater investment in animal disease research to develop new disease detection and prevention strategies. Increases in two-way agriculture require maintaining a high level of trust between trading partners.

Prevention of disease movement in live animals, or animal products, between trading partners requires maintaining an active disease surveillance program to assure trading partners that they are receiving a quality product and that the risk of disease introduction is negligible. Open export markets and new trade agreements force producers to adopt more effective management practices to be competitive. Improved disease control strategies increase profits by lowering costs attributed to disease. This accounts for the large volume of pharmaceutical sales to support effective animal production practices.

Future Needs in Animal Health Research

These are exciting times for animal health researchers. Never before in history have so many new approaches been available for developing better methods of maintaining animal health. Molecular biology and the advent of high-throughput genome sequencing open a window into the understanding of how genes (of microbes and their infected animal hosts) are involved in disease. Genomics will allow us to develop a better understanding of the molecular mechanisms responsible for disease processes and will identify novel approaches to develop vaccines and immuno-modulatory strategies for animal diseases. Animal health research will provide scientific knowledge for the control or elimination of animal diseases, thus optimizing animal production systems to provide abundant, safe, high-quality, wholesome, and nutritious food—food that can successfully compete in global markets. Animal health research also provides solutions that help to prevent development of non-tariff trade barriers. This is an important time to invest in animal health research to reap the benefits of more efficient technologies for production agriculture and to thus maintain the competitive edge for U.S. animals and animal products in a global marketplace.

Animal Health Program Locations

Poultry Production and Products Safety Research
Ann Donoghue, Research Leader
Rm. O-303, POSC
University of Arkansas
Fayetteville, AR 72701
Phone: (479) 575-2413

Western Regional Research Center
Foodborne Contaminants Research Unit
Mark Carter, Research Leader
800 Buchanan St.
Albany, CA 94710
Phone: (510) 559-5984

Center for Medical, Agricultural and Veterinary Entomology
Peter Teal, Research Leader
1600 S.W. 23rd Dr.
Gainesville, FL 32608
Phone: (352) 374-5788

Southeast Poultry Research Laboratory
David Swayne, Research Leader
934 College Station Rd.
Athens, GA 30604
Phone: (706) 546-3433

National Animal Disease Center
Keith Murray, Center Director
P.O. Box 70
2300 Dayton Rd.
Ames, IA 50010
Phone: (515) 663-7201

Avian Disease and Oncology Research Laboratory
Aly Fadly, Research Leader
3606 East Mount Hope Rd.
East Lansing, MI 48824
Phone: (517) 337-6829

Poultry Research Laboratory
Scott Branton, Research Leader
P.O. Box 5367
Mississippi State, MS 39762
Phone: (662) 320-7478

Animal and Natural Resources Institute
Tom Sexton, Center Director
10300 Baltimore Blvd.
Bldg. 200, Rm. 217, BARC-East
Beltsville, MD 20705
Phone: (301) 504-8431

Plum Island Animal Disease Center
Luis Rodriguez and Dan Rock
Research Leaders
P.O. Box 848, Greenport, LI
Orient Point, NY 11944
Phone: (215) 233-6593

Southern Plains Agricultural Research Center
Wayne Ivie, Center Director
2881 F&B Rd.
College Station, TX 77845
Phone: (979) 260-9372

Poisonous Plant Research Unit
Lynn James, Research Leader
1150 East 1400 North
Logan, UT 84341
Phone: (435) 752-2941

Animal Disease Research Unit
Don Knowles, Research Leader
3003 ADBF, WSU
P.O. Box 646630
Pullman, WA 99164
Phone: (509) 335-6022

Arthropod-Borne Animal Diseases Research
Richard Mayer, Research Leader
AG Bldg., Rm. 5031, Dept. 3354
1000 E. University Ave.
Laramie, WY 82070
Phone: (307) 766-3609

Animal Health Program Locations